The Ventilatory Response to Hypoxia in Mammals: Mechanisms, Measurement, and Analysis

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Teppema LJ, Dahan A. The Ventilatory Response to Hypoxia in Mammals: Mechanisms, Measurement, and Analysis. Physiol Rev 90: 675–754, 2010; doi:10.1152/physrev.00012.2009.—The respiratory response to hypoxia in mammals develops from an inhibition of breathing movements in utero into a sustained increase in ventilation in the adult. This ventilatory response to hypoxia (HVR) in mammals is the subject of this review. The period immediately after birth contains a critical time window in which environmental factors can cause long-term changes in the structural and functional properties of the respiratory system, resulting in an altered HVR phenotype. Both neonatal chronic and chronic intermittent hypoxia, but also chronic hyperoxia, can induce such plastic changes, the nature of which depends on the time pattern and duration of the exposure (acute or chronic, episodic or not, etc.). At adult age, exposure to chronic hypoxic paradigms induces adjustments in the HVR that seem reversible when the respiratory system is fully matured. These changes are orchestrated by transcription factors of which hypoxia-inducible factor 1 has been identified as the master regulator. We discuss the mechanisms underlying the HVR and its adaptations to chronic changes in ambient oxygen concentration, with emphasis on the carotid bodies that contain oxygen sensors and initiate the response, and on the contribution of central neurotransmitters and brain stem regions. We also briefly summarize the techniques used in small animals and in humans to measure the HVR and discuss the specific difficulties encountered in its measurement and analysis.
I. INTRODUCTION

The independent discovery of oxygen by the Swedish apothecary Carl Wilhelm Scheele in 1772 and the English scientist Joseph Priestley in 1774 is considered one of the greatest scientific discoveries (for a historical overview, see Ref. 698). To date, 235 years later, it is a well established fact that aerobic metabolism in mammals relies on sufficient oxygen availability in the mitochondria where its reduction to water is coupled to the generation of ATP molecules. To be completed, this process of oxidative phosphorylation requires efficient transport of electrons between five major protein complexes (mitochondrial electron transport chain, attached to the inner mitochondrial membrane). The total amount of oxygen in the body is low (~1.5 liter in total, ~50 ml of which is stored in the tissues), so an undisturbed and complete oxidative phosphorylation requires a continuous fresh supply of oxygen from the environment. Thus an insufficient supply of oxygen may compromise the electron transport chain resulting not only in a decrease in ATP production but also in an increased generation of reactive oxygen species (ROS), such as superoxide anion. ROS can cause structural damage to nucleic acids, proteins, and lipids. At the same time, cytoplasmic signaling-transduction cascades can directly be influenced by ROS. Mobilization of antioxidant defense mechanisms, such as superoxide dismutase that catalyzes the conversion of superoxide anion into hydrogen peroxide and catalase that accelerates the conversion of H₂O₂ into water and oxygen, helps to restore the cellular redox balance.

Apart from cellular antioxidant enzymes, the body has several means to compensate for an acute decrease in oxygen availability (acute hypoxia). Animals relying on pulmonary ventilation for their oxygen supply increase their ventilation to improve the uptake of oxygen. This ventilatory response to hypoxia (HVR) in mammals, i.e., the increase in pulmonary ventilation that results from a decrease in the partial pressure of oxygen in the arterial blood (PaO₂) is the subject of this review. Another means that mammals can utilize to preserve oxygen homeostasis in acute hypoxia is to optimize and decrease the rate of aerobic metabolism, thus reducing oxygen demand and at the same time increasing ATP production from anaerobic metabolism (glycolysis).

Neonatal and adult animals use the options of changing ventilation and/or metabolism in a different way. Apart from an initial fast and transient rise in ventilation, neonates, when exposed to acute hypoxia lasting 20–30 min, display only a minor increase in ventilation but an appreciable reduction in metabolism, thereby optimizing the ratio ventilation over oxygen consumption. During maturation into adulthood, this composite response gradually develops into one with a predominant and sustained increase in ventilation (while the biphasic character of the response is maintained) but without a(n) (appreciable) decrease in metabolism.

Fetal animals respond differently to hypoxia. They display breathing movements in utero that can be detected in an early stage of gestation (for example, ~40 days in lambs with a term duration of ~147 days and ~10 wk in humans). When the fetus is exposed to a low maternal PaO₂ or umbilical cord occlusion, breathing movements are reduced in frequency and can be completely abolished (54, 137, 170). This review describes the mechanisms underlying both the fetal and neonatal breathing responses to hypoxia and summarizes how the neonatal response gradually matures into the adult phenotype. The influence of the genotype on the HVR is also briefly discussed.

Both in neonates and in the adult, the initial increase in ventilation in hypoxia is initiated by the carotid bodies, located at the carotid bifurcations and containing oxygen-sensitive cells that increase their activity in hypoxia (278, 280). The carotid bodies send their afferent input to the brain stem where further processing by the respiratory centers takes place. During the last two decades, considerable progression has been made in elucidating the mechanism of oxygen sensing in the carotid bodies, the factors that modulate it, and the way in which the brain stem processes the afferent traffic from the carotid bodies, and in this review we present a state of the art of these areas.

Studying the mechanisms underlying respiratory adjustments (rise in ventilation, increase in the HVR) to chronic hypoxia and chronic intermittent hypoxia in animals (mainly rats) has revealed a fascinating picture of numerous plastic morphological, biochemical, and functional changes in the carotid bodies and brain stem that are initiated and maintained by transcription factors that regulate the expression of many genes. Both during neonatal development and in adulthood, the respiratory systems of rats and other species display plasticity, i.e., the ability to learn from previous experience and to alter the phenotype of several reflex responses. One developmental period is of particular interest: immediately after birth, within the period of postnatal maturation, there is a critical period (“critical time window”) during which environmental factors can modulate structural and functional properties of the respiratory system. These plastic changes can be permanent and may be irreversible in adulthood and possibly even in adolescence (115). An example is chronic exposure to hyperoxia during this period: it leads to degenerative changes in the carotid bodies and to a reduced HVR that may persist until adulthood (189, 220, 838). Adaptations of the HVR to (intermittent) chronic hypoxic paradigms in this critical neonatal time window will be compared with those in adulthood and placed in the context of plastic changes in the carotid bodies and brain stem that result.
II. THE HYPOXIC VENTILATORY RESPONSE IN FETAL AND NEONATAL MAMMALS

A. Fetal Breathing Movements

Fetal breathing movements (FBM) are coordinated rhythmical contractions of respiratory muscles that intermittently reduce intrathoracic pressure and cause amniotic fluid movements within the trachea (302). FBM are crucial for fetal lung growth and maturation and are observed in all mammalian species studied so far (49, 85, 302, 357, 391, 651, 725, 814). The most commonly observed FBM are rapid irregular movements and can be detected in sheep (mean term duration 147 days) from ~40 days gestation and in humans from ~10 wk (88, 137, 170, 302). Early in gestation, until about day 115, fetal lambs show continuous FBM that are sometimes interrupted by apneas; in the third semester, EEG activity starts to differentiate between states of low- and high-voltage cortical activity associated with rapid eye movements (active sleep) and active movements of neck and limb muscles, respectively. From this developmental stage until term, FBM are limited to active sleep (low voltage cortical activity; reviewed in Refs. 85, 170, 302, 317, 357, 625, 814). This association of FBM and electrocortical activity is disrupted after brain transection rostral from the caudal hypothalamus; when this transection is performed at upper pontine level, i.e., a region close to the inferior colliculus in the vicinity of the principal sensory and motor nuclei of the trigeminal nerve, FBM become almost continuous (170, 171, 271, 363, 396, 523, 814, 815). Sleep state should be considered as a factor modulating fetal breathing rather than providing a primary inducing role: in fetal lambs, periodic FBM precede the electrocortical differentiation by ~1 wk, while at term the animal starts to breathe continuously without changes in EEG pattern (651).

B. Hypoxia Inhibits Fetal Breathing Movements

In fetal sheep, hypoxia caused by (gradually) exposing ewes to a hypoxic gas mixture (mostly with CO2 added to maintain isocapnia) to achieve fetal saturation levels of ~30% leads to adaptations such as spinal inhibition, a reduction in rapid eye movements, facilitation of high-voltage electrocortical electroencephalogram (EEG) activity, inhibition of FBM, a fall in metabolic rate, and a rise in cerebral blood flow (CBF) (85, 88, 137, 170, 317, 408, 588). In the lamb, FBM account for ~30% of overall oxygen consumption (670). Anemia, reduced uterine blood flow, and/or umbilical cord occlusion also lead to FBM inhibition (74, 87, 328, 830). Hypoxic inhibition of FBM also occurs in fetal rat (391), rhesus monkeys (488), and given the relative low FBM incidence in growth-retarded fetuses, presumably also in humans (54, 251). The mechanism of hypoxic inhibition of FBM is complex and may be causally related to one or more of the above adaptations. Below we discuss some major factors influencing hypoxic inhibition of FBM with emphasis on those that may still contribute to shaping the HVR in newborns and even adults.

1. CBF and metabolism and hypoxic inhibition of FBM

The fetal brain exhibits a relatively large rate of oxygen consumption that is tightly linked to oxygen delivery (588). In the fetus, hypoxia not only leads to decreases in neuronal activity and metabolic rate, but also to a rise in CBF, and both these responses have been linked to the inhibition of FBM (588).

2. Pontine and thalamic areas and hypoxic inhibition of FBM

In fetal lambs, brain stem transection at the (mid) collicular level or electrolytic lesions somewhat more caudally in the upper lateral pons in the vicinity of the trigeminal sensory nuclei involving parts of the medial parabrachial and Köllikker Fuse nuclei has two important consequences: 1) it leads to continuous breathing in normoxia and to a dissociation between EEG activity and breathing, and 2) it converts the inhibitory response to hypoxia in the intact condition into a stimulatory one after transection (170, 171, 271, 363, 396, 523, 814, 815). Transection more rostrally through the caudal hypothalamus did not affect hypoxia-induced inhibition of FBM, indicating that an area caudally from it is able to elicit this response independently from higher centers (170).

In fetal lambs, hypoxia facilitates a high-voltage electrocortical EEG pattern (80). FBM and low-voltage electrocortical activity are closely linked, so brain areas influencing behavioral state may modulate hypoxic inhibition of FBM. One such area is the parafascicular nucleus of the thalamus. Lesions in this area abolish hypoxia-induced inhibition of FBM while local electrical stimulation effectively decreases respiratory frequency (397–399).
3. Peripheral chemoreceptors and hypoxic inhibition of FBM

In fetal sheep, the carotid bodies are active and respond to hypoxia with excitation (79). Thus the question arises whether they may have a counteractive role in hypoxia or, alternatively, stimulate brain regions that are directly involved in inhibiting FBM. Carotid sinus nerve and aortic transection do not prevent hypoxic inhibition of FBM but delay the onset of the response, suggesting a supportive but not indispensable influence of the peripheral chemoreceptors (406). After midcollicular transection, hypoxia induced an increase in FBM in fetal lambs, and this response still existed after subsequent carotid body denervation (171). After carotid sinus nerve transection, some fetal sheep showed continuous breathing in normoxia and even FBM in hypoxia; peripheral chemodenervation alone neither changed the incidence of FBM in normoxia nor the hypoxia-induced inhibition of breathing (523). In another study, hypoxic stimulation of FBM after transection depended on carotid sinus nerve integrity, suggesting that in the intact condition (i.e., without any lesion) a stimulatory influence of the carotid bodies may be overridden (363, 396). In general, the emerging picture is that at least in fetal lambs intact carotid bodies are not required to produce hypoxic inhibition of FBM, while their role in stimulating breathing in hypoxia after brain stem transection is somewhat controversial.

4. Placental factors and hypoxic inhibition of FBM

By itself, the fact that umbilical cord occlusion leads to hypoxia in the fetus and decreases the incidence of FBM (328, 830) does not exclude placental factors as the source of FBM inhibition during maternal hypoxemia. Theoretically, a reduced release by the placenta of stimulatory agents could contribute to the reduction of FBM, but to date, such factors have not been identified. The placenta produces inhibitory substances such as prostaglandin E2 (PGE2), adenosine, and several peptides (16, 704, 815), while indomethacin and adenosine antagonists induce continuous breathing and reverse hypoxic inhibition of FBM, respectively (125, 170, 403, 404, 407). After prolonged reductions in uterine blood flow, plasma PGE2 concentration in fetal lambs and the decrease in incidence of FBM showed parallel time courses (328). However, because substances released from the placenta during maternal hypoxemia did not reduce FBM when the fetal Po2 was kept normoxic (413), we suggest that in this condition hypoxic FBM inhibition is caused by local factors in the hypoxic fetal brain that also inhibit respiratory movements during reduced uterine blood flow/umbilical cord occlusion.

5. Neuromodulators and receptors in hypoxic inhibition of FBM

A) ADRENERGIC MECHANISMS. Hypoxia-induced inhibition of FBM may be mediated via adrenergic receptors. In fetal sheep, hypoxia activates catecholaminergic neurons in subcoeruleus- and Kölliker-Fuse regions in the dorsolateral pons, some of which appear to have direct connections with the phrenic motoneuron pool in the cervical cord (551). In both intact and brain stem transected fetal lambs, systemic clonidine reduced the incidence of FBM, and this effect was blocked by an a2-adrenergic antagonist (32). Prolonged reduction in uterine blood flow increased plasma norepinephrine concentration in fetal lambs (328), while ventricular administration of clonidine reduced the incidence of breathing and a specific a2-adrenergic receptor antagonist abolished hypoxic inhibition of FBM (31). Systemic administration of both a1- and a2-adrenergic antagonists had similar effects (270).

B) ADENOSINE A1 AND A2 RECEPTORS. An important molecule in the coupling of cerebral blood flow to metabolism is adenosine. Via adenosine A2 receptors, this molecule induces ~50% of the hypoxia-induced rise in CBF, while A1 receptors mediate hypoxic depression of cerebral metabolism (83, 479, 588).

Experimental evidence indicates that adenosine is an important player in the hypoxia-induced reduction in FBM incidence. In fetal lambs, systemic adenosine caused a decrease in FBM incidence similar to hypoxia, while adenosine receptor antagonists had the reverse effects (76, 125, 405). The posteromedial thalamus displays increased adenosine production during hypoxia and contains a high density of adenosine A2A receptors that mediate FBM inhibition (402, 404, 830, 862). Intra-arterial infusion of a specific A2A but not A1 receptor antagonist abolished both the hypoxic inhibition of rapid eye and breathing movements, while A1 antagonism only eliminated the FBM response (403). Anemic hypoxia upregulates pontine adenosine A1 receptors in the brain stem of fetal lambs, while the same receptor type in the rostroventrolateral medulla is also involved in the hypoxic FBM response (76, 396), possibly by directly depressing local metabolism (84, 479).

The way in which adenosine causes hypoxic inhibition of FBM may be quite complex. Following lesion of the upper lateral pons in the vicinity of the trigeminal sensory nucleus (see above), normoxic respiratory frequency substantially decreases (363). Thus this region may exert a tonic excitatory effect on ventrolateral medullary respiratory neurons in normoxia, possibly by hyperpolarizing postsynaptic neurons. If during hypoxia adenosine, by a presynaptic action, inhibits these pontine neurons, postsynaptic neurons may now depolarize, thus inhibiting respiratory motor output. This would be consistent with in vitro data from neonatal rat and mice.
showing hypoxia-induced depolarization of postinspiratory neurons (see references in Ref. 73).

In summary, ample evidence thus far indicates involvement of adrenergic and purinergic, adenosine A1 as well as A2A mechanisms in the hypoxic inhibition of FBM, but further studies are necessary to further characterize and localize other transmitters and receptor subtypes involved.

6. Some further considerations on hypoxia and fetal breathing

In most of the above studies, hypoxia was tested in fetal lambs in the last gestational semester. In midgestation, hypoxic inhibition of FBM is milder if not absent, indicating that it develops over the second half of pregnancy (496).

Does the low fetal arterial P O2 (<25 Torr) imply a tonic reduction of FBM? This seems unlikely, since neither fetal lambs (125–130 days gestation) nor normal human fetuses show significant increases in the incidence of FBM during maternal hyperoxia (54, 78, 88, 654 and references therein), although at least in the lamb it cannot be excluded that after >130 days of gestation a response of continuous breathing to hypoxia starts to develop (24). In humans, it is only in growth-retarded fetuses that hypoxia results in a higher FBM frequency (54, 251).

In fetal lambs, prolonged hypoxia (>12 h) initially leads to a reduction in FBM frequency, but after a period of 12–20 h it returns to normal (86, 328, 401, 496, 831). How this reversal occurs is unknown, but it has been related to a downregulation of adenosine receptors and plasma glucose levels, respectively (328, 405).

Several alternative mechanisms to explain the hypoxic inhibition of FBM have been suggested, of which acidemia (647), increased blood flow through the ductus venosus (217), and decreased mitochondrial ATP production (407) warrant further investigation. The cellular basis of the response is unknown.

C. Maturation of the HVR in Neonatal Animals

1. Measuring the HVR in small animals

The most frequently used techniques to study breathing in awake mice and rats are modifications of body plethysmography described by Drorbaugh and Fenn (193). Their barometric method is based on the principle that when an animal in a closed chamber inhales a tidal volume, this volume is warmed to body temperature, saturated with water vapor giving rise to pressure changes in the chamber. As discussed by others, both closed and open systems are subject to limitations (e.g., related to pressure changes due to warming, humidification, and air leaks) and particularly in neonatal animals the pressure signal may be contaminated by airway resistance effects that may lead to erroneous estimation of tidal volume (218, 219, 356, 477, 751).

The absence of blood gas data (in most cases) combined with the impossibility to control end-tidal gases renders a reliable comparison of HVRs between treatments difficult. By sheer necessity, the ambient O2 fraction is often taken as the independent variable, but its relationship with the actual stimulus is uncertain and may vary. If in a chronic paradigm normoxic ventilation increases, arterial P O2 will be higher and PCO2 lower for any inspired P O2 level resulting in less carotid body stimulation; thus, in such cases, it is inaccurate to quantify the HVR as the magnitude of the change in ventilation per given decrease in ambient P O2. In addition, a lower initial PCO2 may tend to further reduce at least the early phase of the HVR, owing to O2-CO2 interaction in the carotid bodies (see sect. v). Without addition of CO2, hypoxic tests may be poikilocapnic depending on changes in ventilation and/or metabolism. In the ideal case, preferably three or four measurement points well within the curvilinear part of the exponential ventilation-P O2 relationship should be chosen, avoiding levels at which maximal ventilation is reached (39). A further point concerns the influence of the vigilance state on the HVR (421, 472, 729). Particularly in neonates, EEG recordings are challenging and often not performed, so vigilance state-dependent changes in sensitivity cannot be accounted for. On the other hand, the influence of the latter factor may be limited because sleep-wake cycles are not clearly defined in early life. Balbir et al. (29) proposed a noninvasive method for sleep-wake assessment in neonatal mice, providing a potential means to account for (changes in) brain state. These authors found a sound correlation between high electromyogram (EMG) activity (associated with the awake state) and coordinated limb/head movements and suggested that this behavioral index could be useful in avoiding the stress related to EMG instrumentation in newborn animals (29).

These are only a few of the considerations to take into account when comparing HVRs between treatments.

2. Maturation of the HVR in neonatal animals

In neonatal animals, the HVR appears as a biphasic response consisting of a short initial rise in ventilation (augmentation phase) rapidly followed by a secondary roll-off mostly to a level below control (depressing phase; Ref. 73). Postnatal maturation of the HVR was already described in older studies and in various species such as lambs (e.g., Ref. 102), cats (89, 90, 301, 487, 653), piglets (212), and rats (208, 453, 454). In all species, the relative magnitudes of both phases are subject to maturation. Generally, shortly after birth, the initial rise in ventilation is (much) smaller than the secondary roll-off, rapidly resulting in a lower baseline ventilation compared with
normoxia. However, when maturation proceeds, the initial rise becomes larger, the secondary depression becomes smaller, and eventually a sustained rise in ventilation remains. Age-matched comparisons between species are complicated by the different stimulus paradigms employed. For example, deeper hypoxia may result in a greater initial increase and a larger decrease in ventilation in the depressing phase (see Ref. 73 in which data from humans, rat, cat, monkey, and sheep are shown). In addition, relatively mature species at birth have a better sustained neonatal HVR than those born less mature, e.g., sheep (mean term duration 147 days) are more mature than rat (term duration 18–23 days) and cat (term duration 58–64 days; Refs. 73, 529). In 2- to 17-day-old piglets (term duration 112–120 days), the secondary roll-off seems to be limited to the phrenic motoneuron pool, since upper airways muscle EMGs showed a sustained increase in activity in the presence of a biphasic response of the diaphragm (490).

In most neonates, a vital component of the hypoxic response is a decrease in metabolism (hypoxic hypometabolism; Refs. 90, 529, 531, 532). Newborns of many species demonstrate a drop in oxygen consumption (\(V_{\text{O}_2}\)) that in relatively mature species tends to be smaller. All species investigated show an increase in the ratio expired ventilation (\(V_{\text{E}}/V_{\text{O}_2}\)) (i.e., the ratio expired ventilation over \(O_2\) consumption), indicating a hyperventilatory response, and this occurs irrespective of the magnitude of the HVR (529, 531, 532). Thus ventilation may decrease in hypoxia, but still the outcome will be hyperventilation because of a larger drop in \(V_{\text{O}_2}\). The fall in metabolism is due to a reduction in nonshivering thermogenesis by brown adipose tissue, rather than to the hypoxia-induced (limited) decrease in body temperature (526, 529, 531, 532). Resting metabolism is an important determinant of the hypoxic drop in \(V_{\text{O}_2}\). Generally, animals with higher metabolic rate (e.g., small animals) demonstrate larger decreases in \(V_{\text{O}_2}\), but there are exceptions (243, 526, 531). In a cold environment, requiring more thermogenesis to generate heat, a given animal will show much larger hypoxia-induced decreases in \(V_{\text{O}_2}\) and ventilation (or smaller rise in ventilation) than at an ambient temperature close to thermoneutrality (526, 531). Consequently, measurements of the HVR per se are more meaningful when combined with \(V_{\text{O}_2}\) measurements and supplemented with end-tidal \(CO_2\) values. Furthermore, comparisons between treatments should preferably be made at equal ambient temperature (526, 531).

When maturation proceeds towards the adult stage, hyperpnea replaces hypometabolism as the predominant feature of the hypoxic response, but in many adult species, depending on the ambient temperature, the drop in metabolism can remain an important element of it (243, 260, 482, 526, 531, 675). The magnitude of the fall in metabolism is inversely related to resting \(V_{\text{O}_2}\) relative to body weight and therefore could be limited or absent in adults of larger species (243, 531). The processes underlying maturation of the HVR are briefly reviewed below considering the augmentation and depressing phases separately. In section III, we briefly discuss a potential neural circuit that may be involved in the thermogenic component of the HVR.

A) AUGMENTATION PHASE OF THE HVR. In newborn lambs, piglets, and rats, the relatively rapid initial rise in ventilation in acute hypoxia, designated as the augmentation phase (73), is abolished or greatly attenuated after carotid sinus nerve transection, indicating that the carotid bodies mediate the augmentation phase (103, 166, 249, 473, 474, 533, 740). Shortly after birth, in newborn lambs, the \(P_{\text{O}_2}\) threshold for hypoxic activation of the carotid body is reset from the low fetal \(P_{\text{O}_2}\) (25 Torr) to ~55 Torr (79, 82). This is followed by a gradual increase in hypoxic sensitivity of the carotid sinus nerve, a shift of the hyperbolic response curve to higher \(P_{\text{O}_2}\) values, a gradual appearance of \(O_2/CO_2\) interaction, and an improved ability to maintain elevated discharge levels during prolonged hypoxia (116, 188, 487, 533, 601). The time course of these developmental changes (a few days to 3–4 wk) varies between species, and the relative maturity at birth determines the time point at which adultlike responses are reached (33, 103, 208, 487, 601, 826). Processes underlying maturation of the augmentation phase appear to be a complex fine tuning between afferent nerve innervation of the carotid bodies involving trophic factors such as brain-derived neurotrophic factor and all elements of the of hypoxic stimulus-transduction cascade including membrane channels, neurotransmitters, receptors, and enzymes (sect. III) and are reviewed elsewhere (26, 94, 191, 258, 611).

These maturational processes must keep pace with developmental changes in the central nervous system, for example, in the nuclei of the solitary tracts (NTS), the first relay station of incoming carotid body afferent input. These processes involve changes in the activity of transcription factors, formation of anatomical connections within the neuronal respiratory circuitry, and alterations in neurotransmitter contents and receptor densities to set the balance between excitatory and inhibitory neurotransmission. For example, during maturation, the density and expression of the glutamate receptor ionotropic N-methyl-D-aspartate 1 (NR1) subunit of the NMDA (N-methyl-D-aspartic acid) receptor in the rat NTS increases, which leads to the upregulation of transcription factors and other proteins that are involved in the hypoxic response (564, 702). Another example of a developmental change in the NTS is the downregulation of platelet-derived growth factor (PDGF) during early maturation (702; PDGF exerts an inhibitory influence on ion currents through NMDA receptors, see also sect. III). Also in the rat, Wong-Riley and Liu (847) have shown shifts in the expression of GABA receptor subunits in the NTS and Pre-Bötzinger

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complex around postnatal day 12 (P12) that were paralleled by ventilatory adjustments at the end of the second postnatal week. Between P12 and P16, the HVR showed a sharp decline to reach a minimum at P13, whereafter it gradually increased to a final magnitude on P20–P21. Body temperature during hypoxia gradually rose during the first three postnatal weeks to also reach the normoxic value at P20–P21 (547), consistent with a lesser contribution of the thermogenic component to the hypoxic response at older age (see sect. IV.C2). Maturation of the mammalian respiratory network is reviewed in References 115, 186, 846.

3. Depressing phase of the HVR

The secondary roll-off in neonates may be associated with pulmonary mechanics, a time-dependent decrease in carotid body activity or sensitivity, impaired central integration of afferent carotid sinus nerve input, decreased metabolism, increased CBF and, similar to the inhibition of FBM, active inhibition by pontine and mesencephalic structures involving inhibitory neurotransmitters (reviewed in Ref. 73). Muscle fatigue is unlikely to play a significant role, since the phrenic nerve response in anesthetized neonatal animals does also show a biphasic pattern, indicating that the secondary roll-off is a neurogenic mechanism (121, 249, 522).

In 5- to 10-day-old rabbit pups, brain stem transection at the midbrain-pontine junction converted the hypoxia-induced depression in controls into sustained stimulation in test animals (491). Focal cooling in the locus coeruleus reversibly abolished the hypoxia-induced depression in 3- to 8-day-old anesthetized lambs (522).

Available evidence also points to a role of the carotid bodies in the depressing phase. The temporal response of the sinus nerve discharge in kittens differed among studies, declining in some (116, 487) but sustained in others (81). In 2- to 3-day-old lambs, a sudden hyperoxic stimulus during a hypoxic exposure had a larger effect after 3 min than after 15 min, suggesting waning peripheral chemoreceptor activity or impaired central integration (117). Carotid body denervation attenuated but not completely abolished most of the secondary roll-off in 0- to 13-day-old rats (249), and in newborn lambs the subcoeruleus area showed increased activation in hypoxia after carotid body denervation only (95).

In neonates, adenosine, serotonin, prostaglandin, GABA, opioids, catecholamines acting at \( \alpha_2 \)-receptors, and other neuromodulators may all be involved in the depressing phase (e.g., Refs. 212, 340, 400, 462, 740; see further references in Refs. 73, 285, 702). The strongest case was made for adenosine, which, similar to the fetus, in neonatal sheep reduces hypoxic ventilation both via adenosine A1 receptors, but mainly adenosine A2 receptors (400). In newborn lambs showing a biphasic phrenic hypoxic response, focal cooling of regions in the locus coeruleus prevented the (secondary) fall in respiratory motor output induced by adenosine and hypoxia, respectively (522).

In 3-day-old piglets, the hypoxia-induced increase in tidal volume waned between the second and fifth minute of hypoxia, but this gradual waning could be prevented by the adenosine antagonist 8-phenyltheophylline; 3-wk-old animals, however, did not show this secondary decrease in tidal volume, whilst the adenosine antagonist did not have any influence on the sustained rise in ventilation (212). In piglets 2–7 days of age, aminophylline prevented the secondary decrease in phrenic activity during hypoxia (121). In summary, the secondary roll-off in neonates may be partly a manifestation of similar mechanisms that induce hypoxic inhibition of FBM in the fetus on which a postnatal influence of the peripheral chemoreceptors is superimposed. Note, however, that in many of the above neonatal studies, the end-tidal \( \text{PCO}_2 \) was uncontrolled. If in small animals the HVR is measured at temperatures below thermoneutrality (which is often the case), a fall in metabolism could well contribute to the secondary roll-off. In these cases, measurements of metabolism and body temperature would be very helpful (260). Other factors such as the vigilance state, the severity of the hypoxic stimulus, and other experimental conditions will also influence the magnitude of the depressing phase in the neonatal period.

D. Developmental Plasticity of the HVR

A definition of developmental plasticity of respiratory control was provided by Carroll (115) as “long-term alterations in the structure or function of the respiratory control neural network caused by experience during pre- or postnatal formation of the respiratory control system.”

Overwhelming evidence has identified the neonatal environmental \( \text{PO}_2 \) as a major factor influencing normal HVR development. Hypoxia and/or hyperoxia around the time of birth and the first few weeks thereafter can disturb normal development of the HVR that may persist until adulthood. The effects of perinatal chronic or intermittent hypercapnia were less frequently studied; in the rat, neither the neonatal nor the adult HVR seems to be affected by neonatal hypercapnic exposures (43, 646).

1. Effects of neonatal chronic intermittent hypoxia

Term and preterm infants in particular are repeatedly exposed to hypoxia due to their irregular breathing pattern with many apneas. Animal studies have now made clear that within a critical time window of neonatal developmental plasticity, chronic intermittent hypoxia can induce life-long alterations in the control of breathing with potential pathophysiological consequences (617, 633, 827). In this context, the critical time window of
neonatal plasticity was defined as a period within the developmental stage in which the organism is uniquely sensitive to environmental perturbations that may alter the ultimate configuration of the neuronal network and associated functions (115, 632). In terms of respiratory control, it may imply that exposure to low or abnormally high oxygen (or CO₂) tensions within this window can lead to life-long perturbations in respiratory control.

The effects of repeated exposures to hypoxia can be quite complex and critically depend on the total duration of an intermittent stimulus, its frequency, the overall number of cycles, and postnatal age which all appear to interact (633, 827). One reason for this interaction is that the HVR is built up of a series of sequential events with different lags, time constants, gains, and recovery times. For example, hypoxia augments carotid body activity, increases the discharge frequency in the carotid sinus nerve that results in the release of neurotransmitters in the NTS, in turn leading to the release of neurotransmitters in the ventrolateral medulla etc., eventually causing a time-dependent recruitment of motoneurons. When a new stimulus is offered while one of the processes initiated by the previous stimulus is still in its recovery time or even (partly or fully) active, it is conceivable that the effects of both stimuli on motor output will differ (827).

To investigate the effect of chronic intermittent hypoxia on developmental plasticity of the HVR, a variety of paradigms with different stimulus magnitude, length, and total duration and number of exposures were used in several species such as mice (197), rabbits (789), piglets (828), and rats (reviewed in Ref. 286). In the rat studies, the age of the animals varied from 0 to as many as 30 days and more, well beyond the critical time window for developmental plasticity (115, 286). The results of these studies in different species are equivocal, varying from a reduced (45, 497, 633, 635, 828 and references therein), unaltered (197, 676) to an enhanced (367, 585, 598) HVR and/or carotid body sensitivity. In the rat, blunting of the HVR was reported to persist at 1 mo after cessation of the stimulus paradigm (635). As shown in neonatal piglets, the background Pco₂ during the hypoxic episodes may have an important influence: a poikilocapnic paradigm (10% O₂) blunted the HVR, but hypercapnic chronic intermittent hypoxia (6% CO₂, 10% O₂) caused an increase (828, 829). In instrumented anesthetized piglets, 11 periods of recurrent hypoxia blunted the phrenic nerve response to acute hypoxia in young (2–10 days) but not older (2–3 and 8–10 wk) animals (509; note however that in all recovery periods, these animals breathed 100% O₂ which may have an effect by itself, see below). In most (but not all) studies, normoxic ventilation increased (598, 631–633) and, depending on the animal’s age at the onset of the hypoxic stimulus, this effect was permanent (the first two postnatal weeks were most efficient in this respect; Refs. 632, 633, reviewed in Ref. 45).

These distinct effects of various specific neonatal chronic intermittent hypoxia protocols on the augmentation phase of the HVR and mechanisms underlying neonatal respiratory plasticity are reviewed elsewhere (45, 115, 515, 631, 632, 827). The effects on the depressing phase have not been studied extensively, but at least in the rat, the secondary roll-off seems to be attenuated and related to increased nitric oxide synthesis in the caudal brain stem (286).

We attribute the above divergence in study outcomes to the considerable variations in the applied stimulus level, cycle length, the amount of hypoxic episodes, and the number of exposure days. Differences in methodology (e.g., plethysmography vs. pneumotachography) and developmental critical time windows between species will also play a role (115). Interpretation problems due to chronic intermittent hypoxia-induced changes in normoxic ventilation and the employment of different methods to normalize data are illustrated by the work of Ling et al. (449) and Reeves et al. (633) in adult and neonatal animals, respectively. The poikilocapnic nature of the experiments is also a confounding factor. As shown in infants, kittens, and dogs, hypoxic hypometabolism can be accompanied by a (sustained) fall in Paco₂ in the depressing phase despite the decrease in ventilation (93, 489, 528, 648, 652).

The problem of measuring in a closed loop configuration can be overcome by working with anesthetized animals or ex vivo carotid body preparations. Rat pups subjected to chronic intermittent hypoxia soon after birth (15 s in 5% O₂ followed by 5 min in 21%, 9 episodes/h during 16 h) showed an augmented ex vivo hypoxic carotid body sensitivity that persisted until adulthood and was associated with hyperplasia of glomus cells (585). Carotid bodies from neonates are much more sensitive to chronic intermittent hypoxia than those of adult animals and the effects in neonatal animals are permanent (585). Recently, Pawar et al. (586) showed that in neonatal rats chronic intermittent hypoxia induced an augmented basal release of endothelin-1 (ET-1) in normoxia, upregulation of ETA receptor mRNA, and an enhanced carotid body sensitivity to ET-1. In another study, a chronic intermittent paradigm led to decreases in both the HVR and isocapnic hypoxic phrenic response (635), which would be more in line with the effect of chronic hypoxia. The paradigm consisted of alternating room air with 10% O₂ every 90 s (12 h/day, during 30 days), raising the question whether after a short period of low oxygen, 90 s is sufficient to completely restore tissue oxygenation to normal, possibly resulting in chronic hypoxia rather than chronic intermittent hypoxia.

In conclusion, recent data from ex vivo carotid body responses indicate an increase in sensitivity by neonatal chronic intermittent hypoxia. Technical limitations and methodological differences are probably responsible for the fact...
that as yet these important results could not be consistently confirmed at the level of minute ventilation. Standardizing protocols, uniform quantification of HVR data (combined with metabolic data), and blood gas measurements may help to reach consensus on the effects of neonatal chronic intermittent hypoxia. Intermittent exposures in which hypoxic episodes follow each other so closely in time that episodic reoxygenation is incomplete or does not occur at all may be interpreted as chronic (see also ref 827).

2. Effects of neonatal chronic hypoxia

Cats, sheep, and rats subjected to hypoxia (10–15% O₂) from birth until 24 h before they were tested at ages varying from 5 days to 10 wk showed a markedly reduced HVR, mainly caused by a depression of the augmentation phase (39, 42, 45, 207, 567, 703 and references therein).

In rats and sheep exposed to neonatal hypoxia for 1–2 wk, blunting of the HVR persisted for at least 5–12 wk (39, 476, 567, 703), which in the rat was sex specific (only observed in males; Refs. 42, 476). The effect of neonatal hypoxia on normoxic ventilation is variable: in some studies, a long-lasting increase was observed (527, 566, 567) while it was absent in others (42, 476, 703). At the age of 12 wk, both male and female rats displayed an impaired ventilatory acclimatization to chronic hypoxia (2 wk in 10% O₂) when they were raised in hypoxia until postnatal day 10 (476).

Initially, in the first weeks of maturation the blunted HVR in chronically hypoxic neonates may be due to a delayed carotid body resetting and/or to an attenuation of the maturation of the O₂-CO₂ interaction (301, 303, 437, 730, 826; further references in Ref. 115). Single fibers of the carotid sinus nerve from rat puppies that were raised in 9% O₂ from the first or second postnatal day until 3–4 wk later, showed a 50% lower peak response to severe hypoxia than controls (186). Both at the ages of 3 and 8 wk, rats exposed to hypoxia for 6 days in their neonatal period had increased carotid body dopamine and norepinephrine levels (721). Glomus cells from neonatal rats reared in 12% O₂ during 1–3 wk demonstrated a reduced rise in intracellular [Ca²⁺] in hypoxia compared with cells from normoxic aged-matched controls, and this impaired maturation partially recovered 1 wk upon return to normoxia (731). Rat puppies born and raised in 10% O₂ for 9–14 days had a substantially reduced HVR and possessed type I cells that displayed a reduced expression of maxi-K channels and failed to depolarize in hypoxia (854). In neonatal and juvenile type I cells from rats cultured in 6% O₂ for 12 days, maxi-K channels were downregulated (354). These studies suggest that the neonatal chronic hypoxia-induced HVR reduction is at least partly due to an impaired carotid body function. Not all data, however, support this because changes in sodium and potassium currents that would tend to increase the excitability of type I cells were also reported (315, 316, 726, 727).

Neonatal chronic hypoxia will also affect or delay normal maturation of the central respiratory neuronal network. For example, exposing newborn rat pups to 6 days of chronic hypoxia resulted in a decreased norepinephrine turnover in the caudal part of the noradrenergic A2 region in the NTS (an important projection area of carotid body afferents) at the age of 8 wk, suggesting long-term effects of the neonatal challenge (721). NMDA receptors in pons and caudal medulla of rats raised in 10% for 4–5 wk from birth were downregulated (248). After they were raised in 13–15% O₂ from birth to 5–10 wk postnatally, anesthetized rats showed normal (i.e., similar to age-matched normoxic controls) isocapnic hypoxic sinus nerve responses, but their HVR remained blunted (207). Adult anesthetized rats, kept in 10% O₂ during their first postnatal week, had a normal phrenic response to isocapnic hypoxia, but their HVR was reduced (43). The authors of the latter two studies suggested that the sustained reduction in HVR after neonatal CH may originate downstream from the phrenic motoneuron pool. Abnormal and/or retarded lung growth may then provide a potential explanation (493). Normal maturation of the HVR is also associated with an age-dependent attenuation of the, centrally governed, depressing phase (see above).

In rat puppies, chronic hypoxia from birth delays this decrease (207). Other adaptations observed after neonatal chronic hypoxia in rats are an increase in hematocrit, decrease in metabolism, and an enlarged alveolar gas exchange surface perhaps tending to compensate for a reduced HVR (527, 531 and references therein). Exposing newborn piglets to hypobaric hypoxia during 3 days caused pulmonary hypertension and prevented the rapid remodeling of the cytoskeleton of pulmonary arterial smooth muscle cells that normally contributes to the postnatal decrease in pulmonary vascular resistance (208).

Taken together, neonatal chronic hypoxia-induced blunting of the HVR has multifactorial causes involving plastic changes in the carotid bodies, the central nervous system, and possibly also lung mechanics and pulmonary vessels.

3. Effects of postnatal chronic hypoxia

Earlier experiments in fetal sheep suggested that brief prenatal hyperoxia would hasten resetting of the carotid bodies explaining the reported increased carotid body O₂ sensitivity in these animals (82). This contrasts with many studies showing a reduced HVR after chronic neonatal hypoxia. For example, kittens breathing 30% O₂ during their first 13 days showed a reduced early-phase HVR right away after this exposure (301). In recent years, much attention was focused on the effects of immediate postnatal chronic hypoxia on the HVR on the longer term, extending to the adult age (reviewed in Refs. 39, 45,
115). Here we will briefly highlight some main findings. The effect of neonatal hyperoxia on adult normoxic baseline ventilation appears variable, with some studies showing an increase and others reporting no change (references in Ref. 45). The consequences for the HVR and hypoxic responses of the phrenic and carotid sinus nerves at the adult age can be summarized as follows: 1) chronic neonatal hyperoxia causes a decrease in hypoxic sensitivity at all these three integration levels that persists into adulthood (71, 246, 449). 2) The critical time window to induce this effect, which is dose dependent and can already be induced by 30% O$_2$, lies within the first 2 wk; longer exposures cause no additional depression (40, 41, 71). 3) The attenuation of the HVR can be limited by alternating the chronic hyperoxia with periodic hypercapnia or by replacing chronic by intermittent hyperoxia, suggesting that intermittently increasing carotid body activity reduces the effect (43). 4) Central (carotid sinus to phrenic nerve) integration remains unimpaired, suggesting that the depressing effect originates from the carotid bodies (450). 5) The carotid bodies show degeneration, hypoplasia, changes in membrane properties, and a reduced number of unmyelinated fibers that altogether lead to reduced O$_2$ sensitivity of glomus cells and the carotid body in vitro (189, 220, 622, 838). 6) Petrosal ganglion neurons also show degenerative changes (loss of tyrosine hydroxylase expression; Ref. 220).

A recent study focused on the time course of alterations in hypoxic responses of glomus cells and carotid sinus nerve afferents from rats exposed to chronic hyperoxia starting on postnatal day 7 and lasting for 1, 3, 5, 8, and 14 days, respectively. On postnatal day 8 (so after 1 day of hyperoxia), the secretory response of glomus cells and the nerve response to hypoxia were increased, but beyond exposure days 3–5, both responses were reduced, and this reduced sensitivity persisted until at least 1 wk after exposure (187). Are these effects reversible once animals have reached the adult age? Bisgard et al. (72) reported that sustained hypoxia at adult age (3–5 mo) only sensitized the carotid sinus nerve when the chronic neonatal hyperoxia did not outlast 2 wk. However, another study showed recovery from the deleterious effects of chronic hyperoxia during the first month after birth at the age of 4 mo (247). An antioxidant rich diet during the neonatal hyperoxia did not prevent an impaired HVR at adult age (44), not supporting ROS-mediated toxicity. Future studies are warranted to further examine the influence of dose and duration of neonatal chronic hyperoxia and how an impaired HVR at adult age might be restored.

E. The HVR in Term and Preterm Babies

In most cases, the HVR in young babies is measured by exposing them to 15% O$_2$, seldom with addition of CO$_2$. Most recordings are from quiet sleep when breathing is predominantly under metabolic control and somewhat less unstable than in active sleep, with less frequent arousals. Control of end-tidal gases is unusual so that the magnitude of the HVR reflects the closed-loop properties of the respiratory system.

With the exception of very small premature infants showing a sustained “fetal-like” decrease in ventilation (15), most term and preterm infants exhibit a biphasic HVR upon exposure to 15% O$_2$ during 5 min, much similar to that in neonatal animals. The secondary fall appears to be due mainly to a fall in frequency while in most cases tidal volume shows a sustained increase, sometimes after an initial overshoot (138, 139, 489, 552, 637, 648, 651, 652). With some exceptions (e.g., Refs. 139, 723) early in development the depressant phase of the HVR is associated with a sustained decrease in end-tidal P$_{CO_2}$ (489, 637, 648), similar to neonatal animals, and this is possibly related to a fall in metabolism (147, 332). During maturation, the decrease in frequency gradually becomes less dramatic, and with advancing age, tidal volume tends to remain elevated (489, 648, 651).

The normal developmental time course of the HVR in humans appears difficult to determine. Breathing in term and especially preterm infants is characterized by irregularities and apneas (625) that have been related to immature brain stem function and connections (318) and also to a relatively low Pa$_{O_2}$ leading to brisk hypoxic responses, hypocapnia, and central apneas (552). Consequently, when occurring within the critical developmental time window, both the resulting chronic and intermittent hypoxia or a combination may hinder a normal development of the HVR. Despite the quite different term durations in rodents and humans, the animal data raise the question as to whether oxygen therapy in the neonatal period may have unwanted side effects with regard to the development of normal O$_2$ and CO$_2$ reflex sensitivities. By itself, preterm birth exposes the newborn to an environment that is hypoxic relative to postconceptional age. Whether this is related to the lower HVR of preterm infants compared with age-matched controls remains to be investigated. The higher prevalence of the sudden infant death syndrome (SIDS) in preterm compared with term babies has been related to relative immaturity of the cardiorespiratory neuronal circuitry resulting in failure of ventilatory and arousal responses (518).

Of practical interest when studying the HVR in both neonatal animals and infants is its dependency on the vigilance state (317, 319, 472, 648, 651, 729). Most HVR data were collected in quiet sleep, but potential influences of arousal are not always taken into consideration (648). In a longitudinal study in healthy term infants, the HVR was reported to mature between 2–5 wk and 5–6 mo of age in quiet but not active sleep, especially in children that did not arouse by the stimulus (15% O$_2$ during 5 min...
or 85% SpO₂, see Fig. 1). Even at the age of 5–6 mo, ventilation did not exceed prehypoxic control after a 5-min hypoxic challenge, and this was considered an immature HVR. However, taking into account that the protocol was clearly poikilocapnic and that the HVR was identical to that after 2–3 mo and very similar to the poikilocapnic HVR in adult humans (cf. Fig. 1) leads us to suggest that maturation of the HVR in term infants may be complete at ~2 mo. Direct assessments of carotid body sensitivity with brief exposures to 100% O₂ (Dejours test; Ref. 13) or by offering alternating breaths containing room air and 16% O₂ (108, 843) pointed to full maturation of the peripheral chemoreflex within the first few weeks and possibly even the first 48 h after birth (13; note, however, that data obtained with Dejours tests should be interpreted with caution because the peripheral chemoreceptors may still display considerable activity in hyperoxia). That in term infants the O₂-CO₂ interaction has been reported to emerge at an age somewhere between day 2 and week 8 (no testing after ~2 wk) is not inconsistent with this picture (723).

III. PERIPHERAL AND CENTRAL MECHANISMS MEDIATING THE HYPOXIC VENTILATORY RESPONSE IN ADULT MAMMALS

A. Carotid Body Mechanisms in Oxygen Sensing

The ventilatory response to hypoxia is initiated by the carotid bodies, with the aortic bodies playing only a secondary role, perhaps with the exception of conditions wherein the carotid bodies are functionally eliminated (278). The carotid bodies, bilaterally located in the carotid bifurcations at the port of the brain circulation, have the highest blood flow-to-metabolism ratio in the body and, both anatomically and biochemically, are extremely complex organs (278, 280 and references therein). Not only do they possess cells specialized in oxygen sensing, but in fact, they appear to be polymodal and sensitive to a variety of stimuli such as pressure, hypercapnia, acidosis, hyperkalemia, hyposmolality, circulating hormones, hyperthermia, hypoglycemia, and numerous pharmacological agents (reviewed in Refs. 416, 558).

The two main cell types in the carotid bodies are glomus (type I) cells, having many similarities with sensory neurons and thought to be oxygen sensitive and sustentacular (type II) cells with a glialike appearance (278). Sensory neurons with cell bodies in the petrosal ganglia send peripheral axons to the carotid bodies that run with the carotid sinus nerve, a side-branch of the glossopharyngeal nerve, and have nerve endings closely oriented to type I cells. Central axons of these sensory neurons enter the brain stem with the glossopharyngeal nerve and terminate in caudal subnuclei of the NTS that have extensive connections with the brain stem respiratory network (references in Refs. 232, 335). Hypoxia is followed by release of neurotransmitters by type I cells that act on the afferent endings of the carotid sinus nerve to increase impulse traffic to the brain stem (278). Neurotransmitter release is the result of a rise in intracellular [Ca²⁺] following depolarization of the membrane potential of type I cells. The mechanisms underlying the entire signal-transduction cascade in type I cells from hypoxia to the release of neurotransmitters have been the focus of numerous studies over the last two decades. This has resulted in the acceptance, albeit not without controversies, of a framework in which hypoxia leads to inhibition of ion currents through potassium channels in the cell membrane resulting in membrane
depolarization, influx of extracellular Ca\(^{2+}\) via voltage-gated (mainly L and P/Q type) Ca\(^{2+}\) channels, and release of neurotransmitters (278, 465, 467, 469, 662, 835, see Fig. 2). Overall, the main focuses of attention were the following: 1) the element(s) that initiate(s) the cascade, in other words the identity of the intrinsic O\(_2\) sensor(s); 2) the cause(s) by which the resting membrane potential changes, the identity of potassium channels involved in this, and the link of these channels with the intrinsic O\(_2\) sensor(s); and 3) the identity of the neurotransmitters that are released by type I cells and excite afferent nerve endings. With regard to the identity of the contributing potassium channels and excitatory neurotransmitters, there seems to be a reasonable consensus, although some aspects remain controversial. These issues are covered by several recent reviews and are briefly referred to below but not discussed in detail. Recent studies on the identity of the O\(_2\) sensor(s), and their link with potassium channels, to be discussed below in more detail, have improved our insight into the way type I cells couple changes in Pa\(_O_2\) to alterations in potassium channel activity.

1. Hypoxia decreases the open probability of potassium channels

It is now widely accepted that hypoxia decreases the open probability of potassium channels. A first report in 1998 showing hypoxic inhibition of voltage-gated potassium channels in rabbit type I cells (466) was followed by numerous other studies, but here we only highlight major findings. Three main categories of O\(_2\)-sensitive potassium channels in type I cells have been described. 1) Channels that produce voltage-gated K\(^+\) currents (Kv channels), of which several isoforms have been suggested to be O\(_2\) sensitive (reviewed in Ref. 470). 2) Large-conductance K\(^+\) channels that are activated by both voltage and intracellular Ca\(^{2+}\) (K\(_{\text{Ca}}\), maxi-K, or BK channels; reviewed in Ref. 594). 3) Voltage-independent two-pore domain background (K\(_{\text{2p}}\)) channels that contribute to the resting membrane potential and of which several isoforms are detected in glomus cells (861): K\(_{\text{2p}}\)3.1 (TASK-1), K\(_{\text{2p}}\)4.1 (TRAAK), K\(_{\text{2p}}\)5.1 (TASK-2), and K\(_{\text{2p}}\)9.1 (TASK-3). TASK-1 (TWIK-1-related acid-sensitive K\(^+\) channel; TWIK channel, tandem pore domain weak inward rectifying potassium channel) is the most likely candidate to operate as O\(_2\) sensitive background K\(^+\) channel (reviewed in Refs. 98, 196, 788). Different species appear to have distinct types of oxygen-sensitive potassium channels. For example, in rabbits, Kv isoforms are the predominant types, but in the rat carotid body, significant roles of both maxi-K and TASK-1 have been demonstrated (98, 278, 594 for further references).

2. Carotid body neurotransmitters

The carotid bodies contain a variety of neurotransmitters that are either stored in vesicles or appear as by-products of enzymatic processes in the cytosol where they can influence the signal-transduction cascade (426, 558). The first category comprises the biogenic amines acetylcholine, dopamine, norepinephrine, serotonin, and histamine; (neuro)peptides such as substance P, endothelins, and angiotensin II; and amino acids such as GABA and the purine ATP (see Refs. 394 and 438 for histamine and Ref. 426 for further references). Nitric oxide (NO) and carbon monoxide (CO) are important representatives of the second category, synthesized with the aid of the heme-containing enzymes NO synthase and heme oxygenase, respectively. For an extensive discussion of the role of all these neurotransmitters and modulators, the reader is referred to several excellent reviews (425, 426, 558, 621, 701, 868). With regard to the overall stimulus-transduction cascade, there seems to be consensus, albeit not without controversy, that hypoxia induces the release of acetylcholine and ATP and that these neurotransmitters are
responsible for the excitation of the afferent nerve endings (350, 559, 644, 645, 799; see Refs. 558 and 868 for further references).

### 3. O₂ sensing in the carotid bodies

Over the last decades two main hypothesis of O₂ sensing have been at the forefront: 1) ion channels, and potassium channels in particular, as the initiators of the transduction cascade, referred to as the “membrane hypothesis”; and 2) heme proteins as oxygen sensors, referred to as the “metabolic” or “mitochondrial” hypothesis (278, 279, 430, 620).

The role of potassium channels in type I cell membrane depolarization is now well established (see above). In excised membrane patches, this hypoxia-induced depolarization is still present, but in the course of time a significant rundown occurs, indicating that O₂ sensing may be a membrane-delimited process requiring cytosolic factors to be preserved for longer time periods (800, 842). Whether or not potassium channels possess structural components required for direct oxygen sensing, many of them appear to be redox sensitive (392, 409, 468, 603), but it is unlikely that hypoxia inhibits the current through these channels by oxidizing or reducing functional channel protein components (reviewed in Ref. 282). Generally, reductants and oxidants decrease and increase, respectively, the open probability of rapidly inactivating O₂-sensitive potassium channels in particular, but the effects on maxi-K channels are more variable (references in Ref. 282).

For several decades, mitochondrial cytochromes were considered potential O₂ sensors providing the link between type I cell metabolism and afferent nerve activity (146). In an in vitro perfused carotid body preparation (559), substrates of HO-2 (heme and NADPH) activated native maxi-K channels. Since increased HO-2 activity generates CO, that by itself activates maxi-K channels, it was suggested that the enzyme acts as an O₂ sensor (844). How CO specifically interacts with maxi-K channels is unknown.

At an age <3 mo, mice deficient in HO-2 (HO-2⁻/⁻) show a carotid body phenotype typical for oxidative stress with enlargement of the organ and downregulation of maxi-K channels and a marked upregulation of tyrosine hydroxylase, suggesting that HO-2 could be a potential O₂ sensor (574, 854). Type I cells isolated from HO-2⁻/⁻ mice have a normal catecholamine secretory response to hypoxia, which may suggest that HO-2 is not a key O₂ sensor and maxi-K channels are not required for a normal secretory response to occur (574). Carotid sinus nerve recordings were not performed in this study, so it remains to be seen whether a normal secretory response also predicts a normal nerve response. Although at the level of type I cells the release of catecholamines is considered a measure of type I cell activation (e.g., Ref. 574), changes in their release do not always mirror alterations of carotid sinus nerve activity (100, 185, 350, 351 and references therein). It is interesting that HO-2⁻/⁻ mice showed a reduced poikilocapnic HVR despite the fact that, compared with wild types, they were challenged with a much more severe hypoxic stimulus because of their lower initial arterial P O₂ (4). So it seems reasonable to conclude that to date the identity of HO-2 as an oxygen sensor remains unsettled. Finally, concerning the participation of maxi-K channels in oxygen sensing, it is worth noting that these channels tend to be activated at a membrane potential more positive than that seen in type I cells. Therefore, in order for the maxi-K channel to be the initial sensor, it would have to be open at or near the glomus cell resting membrane potential.

### 2. NADPH oxidase

Acker and co-workers (2, 3) suggested a role of the membrane-bound extra-mitochondrial enzyme complex NADPH oxidase using NADPH as an electron donor that produces O₂ in proportion to P O₂ and proposed that ROS would activate nearby potassium channels in normoxia but less so in hypoxia when NADPH oxidase activity would be reduced (reviewed in Ref. 3). Several NADPH oxidase isoforms were identified in carotid body cells including those containing the subunits gp91phox and p47phox (references in Ref. 3). Later, NADPH oxidase 4 was identified as one of these isoforms in glomus cells (184, 276). Diphenyliodonium (DPI), an unspecific NADPH oxidase inhibitor, increased basal carotid sinus nerve activity in the rat and inhibited the hypoxia-induced increase in nerve activity (146). In an in vitro perfused carotid body...
preparation from the rat, hypoxia reduced a cytochrome b558 isoform whilst the specific NADPH oxidase inhibitor 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEDSF) increased carotid sinus nerve activity and occluded the hypoxic response (424). Transfection of TASK-1 channels in human embryonic kidney (HEK) cells endogenously expressing NADPH oxidase 4 resulted in colocalization of both in the plasma membrane; overexpression of the enzyme enhanced the hypoxia-induced TASK-1 current inhibition in these cells, but eliminating the enzyme abolished the hypoxic response (439).

In the HEK cell model, the concept of ROS-mediated TASK-1 activation was recently challenged because it appeared to be determined by $O_2$ binding to the heme moiety of the enzyme rather than by the production of ROS (582). Other evidence did not support a crucial role for NADPH oxidase. For example, in both rat and rabbit carotid body cells, specific inhibitors of the enzyme did not affect catcholamine release in normoxia or its increase in hypoxia; in addition, DPI seemed to have effects independent from NADPH oxidase inhibition (561). Compared with wild types, adult mice deficient in gp91phox do not show altered responses of ventilation, carotid sinus nerve activity, potassium currents, or intracellular [Ca$^{2+}$] to hypoxia (306, 310, 667). Very interesting results were obtained from p47phox−−/- mice: 1) type I cells from knockout mice did not show the hypoxia-induced increase in [ROS] that was observed in cells from wild types, but displayed an enhanced depression of potassium currents and a substantial greater rise intracellular [Ca$^{2+}$]; and 2) both the HVR and hypoxic carotid sinus nerve responses in knockout mice were substantially greater than in wild types (184, 310, 678). From these observations it was concluded that hypoxia induces a rise in [ROS] in type I cells with NADPH oxidase as their source and that these ROS modulate (increase) the open probability of (K$_c$) potassium channels, rather than being directly involved in oxygen sensing.

3. NO synthase

At least two NO synthase (NOS) isoforms have been identified in the carotid bodies: 1) NOS-1 (neuronal NOS) located in sensory and autonomic nerve fibers innervating vessels and lobules of glomus cells and 2) endothelial NOS (NOS-3; references in Refs. 109, 426). Aspecific NOS inhibition increases basal carotid sinus nerve discharge and enhances hypoxic carotid body sensitivity and the HVR, whilst NO donors do the opposite (290, 619 for further references). Whether NOS is activated or inhibited during hypoxia is controversial (references in Ref. 426). NOS-1−−/− mice show an enhanced hypoxic carotid body sensitivity and HVR, supporting an inhibitory role of NO (389). NO may modulate carotid body sensitivity by altering blood flow and/or exerting direct effects on type I cells (references in Refs. 109, 426, 619).

4. Mitochondrial complex proteins and complex-derived ROS

The role of ROS in oxygen sensing is the subject of a long debate with many controversial data and different views. Partly, the lack of consensus is caused by the difficulty of measuring ROS, by local differences in their production in various microdomains of the cell (cell membrane, cytosol, nucleus, mitochondria), and by differences between cell types (see Refs. 276, 282 for references).

Generally, inhibitors and uncouplers of the electron transport chain inhibit background potassium channels and cause membrane depolarization, voltage-gated Ca$^{2+}$ entry, and neurosecretion. They also stimulate the carotid bodies and, provided the inhibition of electron flux is complete, occlude the hypoxic response (851). Originally it was thought that hypoxia would reduce cytochrome c activity of complex IV of the electron transport chain, which then would cause mitochondrial depolarization and Ca$^{2+}$ release (194). Later the rise in intracellular [Ca$^{2+}$] appeared to depend on extracellular Ca$^{2+}$, and the role of mitochondria as oxygen sensors lost support (99, 465, 802). In addition, several blockers of the electron transport chain (rotenone in particular) have nonspecific effects on potassium channels (573).

A modulating role of mitochondrial ROS, and in this context the influence of cellular redox state on oxygen sensing was investigated by Gonzalez et al. (281). The cellular redox state was assessed by measuring the ratios of reduced (GSH) to oxidized glutathione (GSSG), from which the redox potential ($E_{GSH}$) can be calculated. $E_{GSH}$ is considered the main cellular buffer of ROS and representative for the general redox environment of cells (281). Briefly, Gonzalez et al. (281) found the following. 1) In calf and rabbit carotid body cells, hypoxia did not reduce $E_{GSH}$. 2) The antioxidant and GSH precursor N-acetylcysteine increased $E_{GSH}$ in normoxia in these cells without eliciting an increased catecholamine release. 3) N-acetylcysteine also did not alter the enhanced neurotransmitter release induced by hypoxia and high extracellular [K$^+$]. 4) In rat carotid body cells, specific inhibition of mitochondrial complexes I, II, III, and IV all reduced $E_{GSH}$ and, with the exception of the irreversible complex II inhibitor 3-nitropropionate, decreased cellular ATP levels and increased catecholamine release. N-acetylcysteine prevented these changes in redox state without influencing the effects on [ATP] and neurosecretion. 5) The mitochondrial uncoupler dinitrophenol activated rat carotid body cells without affecting [ATP] and $E_{GSH}$ (275, 281, 282, 679).
The conclusion from these studies by Gonzalez et al. (281) is that oxygen sensing in carotid body cells occurs independently from changes in cellular redox state. In fact, this was supported at the level of afferent nerve discharges in superfused rat carotid bodies (666). However, given the fact that N-acetylcysteine restores the redox state but does not prevent ROS production per se, the question now arises whether mitochondrial ROS may play any (indirect) role, for example, by influencing ATP production. As explained in insightful reviews by Gonzalez and co-workers (276, 282), there is much controversy as to whether hypoxia decreases or increases mitochondrial [ROS]. Briefly, these authors provided reasonable arguments why, unlike some other cell lines, type I cells most likely do not increase mitochondrial ROS production in acute hypoxia and why the limited observed rise in cellular [ROS] in type I cells is most likely due to an increased NADPH activity (310). Yamamoto et al. (860) also observed an increase in [ROS]. Maintenance of the redox state in the face of increased ROS production via this pathway would occur by an increased production of NADPH (reducing equivalent) along the hexose monophosphate pathway (276, 310). Mitochondrial inhibitors NADPH (reducing equivalent) along the hexose monophosphate pathway would occur by an increased production of NADPH (reducing equivalent) along the hexose monophosphate pathway (276, 310). Mitochondrial inhibitors with divergent effects on local ROS production all had stimulating effects on type I cells and occluded the hypoxic response to hypoxia, suggesting no crucial role of mitochondrial ROS on O2 sensing (851).

An absence of a direct involvement of ROS and the cellular redox state in oxygen sensing does not preclude a modulating effect of oxidants and reductants. In the model proposed by He et al. (310) and Dinger et al. (184), ROS, generated by NADPH oxidase activity, facilitates potassium channels. In rat type I cells, inhibition of the cytochrome P-450 enzyme system using NAD(P)H as electron donor prevents oxygen-induced inhibition of potassium channels (304). As suggested by Kline et al. (389), a rise in [ROS] could decrease the bioavailability of NO, increasing hypoxic sensitivity. Mice deficient in neuronal NOS have an augmented HVR (389).

5. Individual mitochondrial complexes

To date, the identification of one single mitochondrial complex protein as the oxygen sensor in type I cells has been elusive. Light absorption photometry has identified a cytochrome, a592, as a unique component of carotid body cytochrome-c oxidase (complex IV) with a redox sensitivity that depends on the Po2 in a nonlinear fashion (3, 733). By inducing a shortcut of electron flow within complex IV, this low Po2 affinity cytochrome would contribute to mitochondrial depolarization and intracellular Ca2+ regulation. However, the intracellular [Ca2+] response to hypoxia depends on extracellular, not intracellular, calcium stores (99, 465, 802), whilst the ATP synthase inhibitor oligomycin, despite causing mitochondrial hypopolarization (194), has a similar stimulatory effect on type I cells as mitochondrial complex inhibitors and uncouplers which cause mitochondrial depolarization (851). Despite this, the idea of heme-containing oxygen sensors with different O2 affinities in distinct microdomains of the cell, enabling it to adapt to changes in cellular Po2 profile (“oxygen redirection”), is interesting (837 for references).

In rat PC12 cells, hypoxia produced a selective and fast downregulation of complex I mRNA. This suggested that, apart from being involved in the flow of electrons through the electron transport chain and as such may indirectly contribute to rapid changes in [ATP], complex I may play an important role in adapting mitochondrial respiration to prolonged periods of hypoxia (606). In chronic intermittent hypoxia, carotid body complex I activity also appeared to be reduced (596).

Mice heterozygous for succinate dehydrogenase subunit D (SDHD+/−), one of the anchoring proteins of complex II (607), show mild carotid body hypertrophy and hyperplasia and an enhanced catecholamine release by type I cells in normoxia. These features are associated with a decrease in K+ conductance (due to a decrease Ca2+ sensitivity of maxi-K channels) and a persistent Ca2+ influx; the hypoxia-induced CA release was enhanced in these animals, but not significantly (607). Thus these mice do not seem to support a crucial role of complex II in O2 sensing, and this may fit the finding that the irreversible complex II inhibitor 3-nitropropionate did not alter the catecholamine release and ATP content of rat carotid body cells (275). However, carotid body-sinus nerve preparations from SDHD+/− mice were not studied. Human carriers of a missense mutation within the SDHID gene show an altitude-related phenotype (20). Thus it remains to be seen whether there may exist a relation between complex II activity and oxygen sensing (for further discussion and references, see sect. iv).

6. Mitochondria and the link between metabolism and potassium channel activity

Failure to show an indispensable role of mitochondrial ROS in O2 sensing, and the fact that it has been elusive to identify one of the electron transport chain complexes as the O2 sensor that initiates the entire transduction cascade, does not preclude a mitochondrial role because O2 sensing could be a direct or indirect function of mitochondrial energy metabolism and ATP synthesis (851). Mitochondrial complex inhibitors and uncouplers have variable effects on [ROS], but two effects that they have in common are that they decrease ATP levels and activate type I cells (276, 851), perhaps with the exception of specific complex II blockers (275).

Recently, cellular energy status was linked to cell activity in type I cells via AMP-activated protein kinase
(AMPK), which is considered the master regulator of glucose and fatty acid metabolism (417 and 786 for references). AMPK is a cellular energy sensor and sensitive to the cellular AMP-to-ATP ratio. Metabolic stress including hypoxia increases this ratio and activates AMPK, with the result that catabolic processes are upregulated and anaerobic processes inhibited (417, 786). Recently, Evans et al. (223) proposed an AMPK-mediated mechanism of O₂ sensing in type I cells, and they provided substantial experimental evidence to support their model. Their main findings were as follows. 1) In rat type I cells, they identified an AMPK isoform that is located close enough to the cell membrane to participate in a "membrane delimited" process. 2) In type I cells, the AMPK mimetic agent AICAR (5-aminoimidazole-4-carboxamide riboside) inhibited TASK-1 and maxi-K channels and caused depolarization and increased Ca²⁺ influx whilst also increasing sensory afferent discharge from an isolated carotid body preparation. 3) These effects could be inhibited by the AMPK antagonist compound C that also reduced the effect of hypoxia on type I cells (223, 852, 855). This model is attractive not only because it provides a link between cellular metabolism and potassium channel inhibition but also because it may reconcile the "membrane" and "mitochondrial" hypotheses of O₂ sensing (852).

Other, more direct, links between metabolism and TASK-1 channel inhibition are also possible. In rat type I cells, MgATP and other Mg nucleotides have a powerful stimulatory action on background potassium channels (800). Mitochondrial complex inhibitors cause a rise in [Mg], reflecting a fall in [MgATP]₅, and the channels were suggested to be coupled to (or possess) a low-affinity Mg nucleotide sensor (800). A direct, depolarizing effect of adenosine via A₂A receptors resulting in a rise in [Ca²⁺] in type I cells has also been reported (856). Depletion of MgATP reflects increased production of AMP, providing pathways to increase [adenosine] and activate AMPK, respectively (800).

The idea of carotid bodies as metabolic sensors is not new. For example, in an in vitro preparation, the cat carotid body is excited by inhibition of glycolysis (560). Recent studies have identified type I cells in isolation, both in coculture with petrosal ganglion neurons and in fresh carotid body slices, as direct glucose sensors that depolarize and release neurotransmitters upon lowering of glucose levels (254, 580, 870). However, a direct effect of glucose on type I cells is not uncontroversial because in the rat, low glucose increases spontaneous ventilation, an effect that depended on the integrity of the carotid sinus nerve, while in isolated carotid body-sinus nerve preparations it failed to have any effect on nerve discharge and the release of catecholamines and ATP, respectively (64, 141). Several studies have clearly established the role of the carotid bodies in glucose homeostasis, which can be modulated by GABAergic mechanisms in the NTS (see Refs. 440, 870 and references cited therein). Interestingly, in humans, low plasma glucose was reported to double the HVR, but this could be indirectly mediated by counterregulatory hormones (825).

In summary, various heme proteins, such as NADPH oxidase, HO-2, NOS, and mitochondrial complex proteins may operate in parallel or sequentially as oxygen sensors. The oxygen sensing machinery is already operative in mild hypoxia, in a Po₂ range in which mitochondrial complex proteins, due to their low P₅₀, will not yet be compromised (427, 621). This suggests that more than one O₂ sensor must be operative (380, 621, 800). Across (but also within) animal species, various classes of potassium channels play a crucial role in the membrane depolarization of type I cells in hypoxia by reducing their current. There are multiple ways in which these channels can be inhibited. One of these, changes in the cellular AMP/ATP ratio, has recently been identified as a very sensitive manner to couple cell metabolism to potassium channel activity and appears to operate already well before mitochondrial complex proteins are compromised (415). Type I cells have an extremely complex biochemical content and release numerous substances during hypoxia, of which ACh and ATP are thought to be the principle excitatory neurotransmitters acting at the afferent endings of petrosal ganglion neurons that innervate the carotid bodies.

B. Hypoxia Activates the Transcription Factor Hypoxia-Inducible Factor-1

Chronic hypoxia induces expression of genes encoding factors that 1) promote tissue oxygenation (e.g., angiogenesis); 2) sensitize oxygen-sensing mechanisms and facilitate central processing, thus augmenting the HVR; 3) increase the blood O₂ transport capacity (erythropoietin production); and 4) adapt cellular metabolism by facilitating glucose uptake (upregulation of glucose transporters) and promoting anaerobic metabolism whilst repressing but also optimizing oxidative phosphorylation. This altered expression of these and many other genes is initiated by increased activity of numerous transcription factors (reviewed in Refs. 101, 133, 149, 381, 661, 693), one of which appears to orchestrate this master plan of gene expression: hypoxia-inducible factor-1 (HIF-1), discovered by Semenza and co-workers (see Ref. 689).

HIF-1 is a heterodimer consisting of the constitutively expressed nuclear subunits HIF-1β and HIF-1α. The latter subunit, HIF-1α, undergoes (cytoplasmic) posttranscriptional modification under the influence of the Po₂ (Fig. 3; Ref. 690). HIF-1 induces many target genes encoding proteins that influence O₂ supply, metabolism, transcription factors, and apoptotic and enzymatic processes (101, 133, 371, 693). In euoxic conditions, HIF-1α is both synthesized and rapidly degraded, but in hypoxia it is
rescued from proteasomal degradation enabling it to translocate to the nucleus where it dimerizes with the constitutively expressed HIF-1 to form the transcription factor HIF-1. The dimer binds to the hypoxia response elements of target genes where it recruits coactivator proteins to promote transcription. Structure and properties of HIF-1 and its subunits can be found in References 689 – 691. HIF-1 has two paralogs: 1) HIF-2, which is also regulated by O2, and, when dimerized with HIF-1, promotes the transcription of both overlapping and distinct target genes (references in Ref. 694); and 2) HIF-3, with a largely unexplored function (369, 694). In many if not all body cells, HIF-1 is upregulated during hypoxia.

There are many pathways of posttranscriptional modification of HIF-1 (reviewed in Refs. 133, 369, 692, 694). Here we only briefly discuss the controversial role of ROS.

1. HIF-1α stabilization via increased [ROS]

HIF-1α contains an oxygen-dependent degradation domain with highly conserved specific proline residues (Pro402 and Pro563 in mice, Pro402 and Pro564 in humans) that are hydroxylated in normoxic conditions. This hydroxylation is catalyzed by enzymes containing a prolyl hydroxylase domain (PHD). Currently three PHD family members are known, PHD1, PHD2, and PHD3, of which PHD2 is considered the primary HIF prolyl hydroxylase in normoxia (63, further references in Ref. 369). PHD2 is a dioxygenase that uses molecular oxygen and α-ketoglutarate as substrates, generates CO2 and succinate as by-products, and utilizes Fe2+ and ascorbic acid as cofactors. Prolyl hydroxylation of HIF 1-α is required for binding of the von Hippel-Lindau tumor suppression protein pVHL which targets the complex for ubiquitin-mediated degradation (Fig. 3; Refs. 352, 353, 499). The current view is that during hypoxia the activity of PHD2 is reduced by a combined effect of substrate (oxygen) limitation and inhibition of the Fe2+ catalytic center by ROS possibly produced by complex III of the mitochondrial electron transport chain (55, 122, 123).

2. HIF-1 stabilization via decreased [ROS]

An opposite role of ROS in HIF-1 stabilization was proposed by Huang et al. (341; see Ref. 276 for further references). In human Hela cells, they showed increased stability of the HIF-1α subunit in hypoxia and a rapid breakdown in normoxia. In hepatoma 3B (Hep3B) cells, hypoxia also induced HIF-1α stabilization and an increased expression of erythropoietin mRNA, and both effects were dose-dependently blocked by preexposure to hydrogen peroxide. Oxidizing HIF-1α inhibited HIF-1 stabilization via increased [ROS].

**Figure 3.** Influence of prolyl hydroxylases on hypoxia inducible factor (HIF) stability. In normoxia, with sufficient oxygen availability, prolyl hydroxylases [PHD; PHD2 is the isoform that is considered the primary HIF prolyl hydroxylase in normoxia and utilizes iron and ascorbic acid (asc) as co-factors] hydroxylate crucial proline residues in the degradation domain of HIF-1α. This enables the von Hippel-Lindau protein (pVHL) to bind to these residues and ubiquitylate (ubi) the protein for proteasomal degradation. As a consequence, the nucleus contains low levels of HIF-1α and thus also of HIF-1, the heterodimer of HIF-1α and HIF-1β. In hypoxia, PHD2 activity is limited by low Po2. Functional activity of HIF-1α is regulated by factor inhibiting HIF (FIH) which, in an oxygen-dependent way, hydroxylates a crucial asparagine residue in the COOH-terminal domain (not shown). In hypoxia, HIF-1α is stabilized, activated, and translocated to the nucleus. An important additional step in HIF activation in hypoxia, correlated with the induction of target genes, is the activation of the coactivators p300 and cAMP response element binding protein (CREB) which combine with the heterodimer. This complex binds to the hypoxia response element (HRE) of target genes while additional coactivators are also recruited. αKGD, α-ketoglutarate; succ, succinate; ROS, reactive oxygen species.
DNA binding, and this could be reversed by reducing agents, possibly indicating that hypoxia stabilizes HIF-1α by reducing specific cysteine sulphydryls in the molecule, whilst hypoxia and hydrogen peroxide would reverse this (341 and references therein).

What is the scenario in glomus cells? In carotid bodies from rats exposed to chronic intermittent hypoxia, HIF-1α was upregulated and complex I activity was reduced, whilst [ROS] and hypoxic carotid body sensitivity were increased (597, 600). HIF+/− mice did not display these adaptations, and together these findings would suggest stabilization of HIF-1α by increased [ROS] resulting in the augmented HVR (600; see also sect. vG). However, with the assumption of enhanced ROS production by mitochondrial complex inhibitors, the finding that in type I cells from rat, complex inhibitors significantly reduced HIF-1α upregulation induced by superfusion with a hypoxic solution for ~45 min, would rather support the notion of HIF stabilization with reduced [ROS] (22). This model could also provide a potential explanation for the increase in HVR in humans after chronic treatment with antioxidants (321, 608). Notably, the antioxidant N-acetylcysteine not only augmented the HVR in young subjects but also increased the production of erythropoietin, indicating upregulation of HIF-1 (321). Reduction of the isocapnic HVR by low-dose anesthetics or the carbonic anhydrase inhibitor acetazolamide could be reversed by an antioxidant cocktail consisting of α-tocopherol and ascorbic acid (777, 780, 781). This reversal occurred in the setting of an acute experiment, so presumably it was unlikely to be mediated via increased stabilization of HIF-1α. It was speculated that the reversal could be due to independent, inhibiting effects of the antioxidants on potassium channels, although other explanations are possible (777, 780, 781). The stimulating effect of chronic ascorbic acid on the HVR is hard to reconcile with a single role of this antioxidant as cofactor of PHD2. An effort to eliminate the other cofactor of PHD2, Fe2+, by infusing the iron chelator desferrioxamine (DFO) for 8 h did not alter the HVR in humans (639). This could be due to failure of DFO to penetrate into type I cells, and this is supported by data from in vitro perfused carotid body from the rat (168). Finally, in humans, iron infusion did not influence the augmentation of the HVR that is normally observed after chronic hypoxia (see sect. vF), and this could be due to the inability of iron to reach the interior of type I cells (711).

C. Central Effects of Hypoxia

1. General effects of central nervous system hypoxia on breathing

In the central nervous system (CNS), moderate hypoxia causes a generalized reduction in neuronal excitability and decrease in intracellular [ATP]. Depending on the duration and severity an initial transient alkalosis, an increase in potassium conductance and hyperpolarization may ensue acting to conserve energy. Some neurons, however, depolarize in hypoxia and if some other conditions are also met may be classified as central O2 sensors (see sect. mC2). Severe, permanent hypoxia is generally followed by intracellular acidosis, influx of Ca2+ and Na+, efflux of K+, persisting depolarization, further decreases in intracellular [ATP], and eventually cell death (435). Hypoxia in the brain (central hypoxia) affects breathing in a way that depends on the integrity and activity of the carotid bodies. In addition, several brain regions are claimed to contain oxygen sensors that may play a role in the HVR in some circumstances.

Shortly after carotid body denervation, cats anesthetized with chloroalose (urethane) show decreases in ventilation and phrenic nerve activity with both hypoxemic and CO hypoxia (314, 361, 507). With severe hypoxia, the phrenic neurogram displays a gasplike pattern (507). Animals anesthetized with thiamylal, however, failed to show ventilatory depression immediately after carotid body excision, even with severe hypoxia (710). Carotid body-denervated rats anesthetized with urethane do also show a clear-cut hypoxia-induced decrease in respiratory motor activity (436). A depressant response of CNS hypoxia is also manifest in chloroalose-urethane anesthetized cats subjected to isolated brain stem perfusion with hypoxic blood, even when the PCO2 of the peripheral blood is kept hypercapnic (794, 823). In anesthetized cats subjected to carotid body denervation or artificial brain stem perfusion, hypoxic ventilatory depression may be caused by both a fall in medullary PCO2, the metabolic stress of lactic acidosis and/or decrease in [ATP], as well as inhibitory neurotransmitters (62, 436, 540, 543). The absence of a depression in thiamylal-anesthetized animals (710) might suggest involvement of a GABAergic mechanism.

Artificial brain stem perfusion via one vertebral artery in the cat supplies the pons, medulla, and cerebellum with “central blood” (59) so central hypoxic depression in this preparation must originate from one or more of these regions, presumably pons and/or medulla.

At least in cat and rat, the effect of (severe) CNS hypoxia on breathing differs between the anesthetized and awake states. Awake carotid body denervated cats respond to mild hypoxia (FiO3, ~14%) with mild to moderate hypoventilation (237, 261, 510) or virtually no change (464). Severe hypoxia, however, results in a pronounced tachypnea and decrease in tidal volume resulting in either a reported decrease (261) or increase (510), respectively, in alveolar PCO2. In unanesthetized animals, a transition into unconsciousness during severe hypoxia did not result in any change in breathing pattern, suggesting the diencephalon as the source of the tachypnea (510). Immediately after carotid body denervation, the awake rat may show some hypoxic respiratory depression (492), but the most common response is a limited rise in venti-
lization (570, 664, 665), raising the question whether the carotid body-denervated rat is a suitable model to study the isolated effect of central hypoxia. This is illustrated by the fact that acute hyperoxia leads to an increase in ventilation in this model, indicating a depressant effect of central hypoxia (570). With prolonged central hypoxia, awake goats and dogs undergoing selective carotid body perfusion with normocapnic-normoxic blood showed an increase in ventilation (165, 836) and an absence of respiratory depression (706), respectively. Sleeping dogs increased their ventilation with central hypoxia, but after carotid body denervation there was no response (151). Soon after carotid body denervation, ponies showed no hypoxic ventilatory depression; however, this does not preclude an inhibitory effect of low central PO$_2$ during chronic hypoxia because acute hyperoxia induced hyper-ventilation in these animals (239).

Finally, note that the depressant effect of central hypoxia in anesthetized cats with denervated carotid bodies is different mechanistically from HVD in intact cats, with the latter being initiated by the carotid bodies (see also section V).

A) CENTRAL O$_2$ SENSORS AND HVR. Before a neuron that depolarizes in hypoxia can be classified as intrinsically sensitive to O$_2$, at least four criteria must be met. 1) The depolarization should occur independently from synaptic events. 2) Activation of these neurons is independent from input from the arterial chemoreceptors. 3) Within the range of physiological O$_2$ levels, the sensitivity of these neurons should be much greater than that of other neurons. 4) At least under certain physiological conditions, stimulation by low oxygen of these neurons should be able to elicit an appropriate (cardio)respiratory response. Below we summarize which regions belonging to the respiratory neuronal network are claimed to possess neurons that are intrinsically sensitive to oxygen.

1) Location of central O$_2$ sensors. Exposing anesthetized cats to 10% O$_2$ excited ~20% of neurons studied in the caudal hypothalamus, independently from carotid sinus nerve integrity (183). Ninety percent of these cells had a cardiovascular and/or respiratory rhythm, even after carotid body denervation (183). In brain slice preparations, ~80% of caudal hypothalamic neurons responded to hypoxia independently from synaptic blockade (182, 329). O$_2$-sensitive neurons in the caudal hypothalamus were also found in the rat (330, 671). Oxygen-sensitive cells in the hypothalamus may also reside rostrally in parvocellular cells of the paraventricular nucleus (385).

Located in an area from which O$_2$-sensitive neurons in the caudal hypothalamus can be antidromically activated, the pontine periaqueductal gray (PAG) contains many neurons intrinsically sensitive to O$_2$ (410, 671). Depending on the stimulus location, PAG activation elicits cardiovascular responses that are mediated via the rostroventrolateral medulla (RVLM; references in Ref. 410). In slice preparations, the locus coeruleus in the dorsolateral pons contains many neurons that are inhibited by hypoxia and a smaller percentage that is depolarized, but it is uncertain whether this represents intrinsic O$_2$ sensitivity (545, 864).

Both stimulation of the carotid bodies and central hypoxia activates neurons in the RVLM, and in the latter case, this activation is resistant to local glutamate receptor antagonism and denervation of the carotid bodies. So these cells may be intrinsically O$_2$ sensitive, and this notion was corroborated by the finding that in slice preparations RVLM neurons retained their depolarizing response to hypoxia or cyanide even with synaptic blockade (741, 742, 744, 745). Nolan and Waldrop (553) reported activation of ~64% of neurons studied in the same RVLM area when exposing anesthetized spontaneously breathing rats to hypoxia. Many of these neurons displayed a temporal discharge pattern related to the cardiac and/or respiratory cycle; a similar percentage was activated after bilateral transection of both the vagus and carotid sinus nerves and cervical sympathetic and aortic depressor nerves (553). Brain slices including this RVLM area and from a region projecting to the spinal cord contained neurons the majority (~70–80%) of which was excited by low PO$_2$ even after synaptic blockade; however, a substantial amount (~20%) was inhibited (553). O$_2$ sensitivity in the RVLM is not a unique property of spinally projecting barosensitive neurons because it seems to be widely distributed in this area with many neurons either excited or inhibited by hypoxia (378). Taken together, the caudal hypothalamus, PAG, and RVLM might act as an accessory circuit to amplify ventilatory and cardiovascular responses to hypoxia (410).

Located just rostral to the C1 region of the RVLM, the pre-Bötzinger complex contains oxygen-sensitive cells. Local application of NaN$_2$ into this area augmented inspiratory output including gasping in the phrenic neurogram (718). Dissociated cell cultures with neurons from both the C1 region and pre-Bötzinger complex contained cells of which ~60% responded to NaN$_2$ with membrane depolarization and an increase in firing frequency, but ~40% with inhibition, and these responses were resistant to synaptic blockade (500). Both RVLM regions express HO-2 (501), an enzyme that in the carotid bodies may be involved in oxygen sensing (844; see also sect. viC). The expression of HO-2 in these regions is limited to oxygen-sensitive neurons; blocking enzyme activity abolishes the response of these neurons to hypoxic hypoxia and NaN$_2$ (153).

Oxygen-sensitive cells were also found in the NTS. In coronal slices from the level of the obex, 75% of the neurons tested responded to hypoxia with hyperpolarization, decreased input resistance, and decreased activity, while 25% showed increased activity (583). Hypoxia in these slices also decreased postsynaptic potentials elicited by electric stimulation of afferents in the solitary
tract, suggesting hypoxic gating of incoming afferent input (583). O₂-sensitive neurons in the NTS were also found by Nolan and Waldrop (553).

Finally, the hypoglossal nucleus also contains neurons that depolarize upon exposure to hypoxia (297).

The mechanism of oxygen sensing in brain stem and hypothalamic neurons is largely unknown. Does a uniform O₂ sensor operate that would facilitate identification and localization of these neurons? Some authors have proposed a lack or paucity of ATP-sensitive potassium channels as a potential basis of oxygen sensing (e.g., Ref. 864), which could make sense because hypoxia decreases intracellular [ATP] in the brain (435, 436). Also, several other potassium channel species have been suggested to be involved in central oxygen sensing, as well as various calcium and sodium channels (329, 745, 864; reviewed in Ref. 544). Recently, D’Agostino et al. (153) reported that excitation by hypoxia of neurons in the C1 region and pre-Bötzinger complex of rats critically depended on the activity of HO-2.

2. Role of central O₂ sensors in the HVR?

The contribution of central oxygen sensors to the HVR is unknown. Does the absence of an increase in ventilation shortly after carotid body denervation as observed in cats and dogs imply a trivial contribution of central O₂ chemoreceptors to the HVR of an intact organism? Taking into account that the carotid body may provide oxygen-sensitive neurons in the RVLM with a tonic excitatory input (741, 744), a preparation with denervated carotid bodies would not be the most suitable model to get more insight into this issue. Could the increase in ventilation with isolated central hypoxia in goats and dogs with intact, separately perfused carotid bodies be due to a facilitating peripheral chemoreceptor input, explaining, for example, the elimination of the response in dogs after carotid body denervation (151)? Selectively perfusing the carotid bodies with either hypoxic or hyperoxic blood to subsequently measure the effects of central hypoxia could give some clue. In anesthetized cats and awake goats, the reverse paradigm, i.e., varying the peripher al O₂ while keeping central O₂ constant at different levels, did not reveal an influence of brain stem PO₂ on the ventilatory response induced by carotid body hypoxia (i.e., no peripheral-central O₂ interaction; Refs. 165, 794).

In cats, ponies, and rats, the respiratory system displays a remarkable plasticity in restoring the HVR after denervation of the carotid bodies (70, 492, 664, 710). Rats displayed a complete recovery of the HVR 90 days after carotid body denervation which was temporally related to changes in tyrosine hydroxylase expression in various brain stem areas containing central O₂ sensors, e.g., the A2/C2 region in the caudal NTS and the A1/C1 region in the RVLM (665, 665). Complete HVR recovery on a comparable time scale was also observed in rats after lesioning glutamate receptor-containing neurons in the NTS, initially resulting in a 70% reduction of the response (130). It is tempting to speculate that tyrosine hydroxylase-containing central O₂ sensors could serve as a “back-up” mechanism to detect low oxygen levels in cases where peripheral chemoreceptors are functionally eliminated, but further studies are necessary to confirm this.

Diencephalic structures play a role in the severe hypoxia-induced tachypnea in awake cats with denervated carotid bodies (510). Disinhibition of cortical-hypothalamic interactions by low PO₂ contribute to this phenomenon. Because the caudal hypothalamus contains neurons excited by O₂ independently from the carotid bodies and provide the RVLM with an excitatory input, these O₂ sensors could contribute to it (331, 813). Direct stimulation of the caudal hypothalamus increases respiratory frequency (813).

3. CNS neurotransmitters and HVR

A) GLUTAMATE, GABA, GLYCINE, ADENOSINE, AND ATP. The entire respiratory network participates in the HVR, so it is not surprising that it can be influenced by numerous neurotransmitters/modulators and receptor agonists/antagonists. Respiratory midbrain and brain stem regions, each with a separate contribution to the HVR, contain a variety of neurotransmitters (recently reviewed in Ref. 732). In Figure 4, the major medullary and pontine regions are shown that participate in the HVR. Classical mediators of the HVR are GABA, glutamate, and adenosine, and their temporal profiles and roles were reviewed previously (104, 327, 379, 502, 540, 649, 752). Generally, glutamate is considered an excitatory neurotransmitter in the HVR, while GABA, glycine, and adenosine are inhibitory (18, 104, 327, 379, 565). This is probably an oversimplification, because one particular transmitter or modulator

FIG. 4. Major brain stem regions involved in the ventilatory response to hypoxia. Major cell groups involved in the ventilatory response to hypoxia are shown in coronal sections between rostrocaudal levels of the rat brain stem from Bregma −9.16 to −14.60 mm. 1, A1 noradrenergic region; 2, caudal ventral respiratory group; 3, A2 noradrenergic region; 4, raphe pallidus; 5, raphe obscurus; 6, medial subnucleus of the nucleus of the solitary tract; 7, commissural subnucleus of the nucleus of the solitary tract; 8, rostral ventral respiratory group; 9, C1/A1 adrenergic/noradrenergic region; 10, C2 adrenergic region; 11, C1 adrenergic region; 12, pre-Bötzinger complex; 13, Botzinger complex; 14, raphe magnus; 15, retrotrapezoid nucleus; 16, A5 noradrenergic region; 17, locus coeruleus; 18, parabrachial nucleus, medial part; 19, Kölliker-Fuse nucleus. Py, pyramidal tract; scp, superior cerebellar peduncle; Sol, nucleus of the solitary tract; V, motor nucleus of the trigeminal nerve; VII, facial nucleus.

[Sections redrawn from Paxinos and Watson (587).]
can have distinct effects on the HVR, depending on the dose, route of administration, and site of application in the CNS. For example, in rats, at an ambient temperature of 30°C, the GABA agonist muscimol reduces the HVR when microdialyzed into the NTS but augments it when applied in raphe magnus (539, 772). Recently, a modulatory (facilitatory) role for ATP has been described, in which it is released in ventral medullary surface areas in the secondary phase of the HVR and possibly acts in the Pre-Bötzinger complex to increase respiratory frequency via activation of metabotropic (P2Y) receptors (284, 471).

Below we summarize the most relevant data, mainly collected over the last decade, concerning the important role of monoamines (with emphasis on serotonin) and NO in the HVR. Figure 4 can be used as a reference to envisage the different brain stem regions where these transmitters exert their action.

**B) Monoamines and the HVR.** The absence of a significant effect of catecholamine depletion on the HVR may suggest that norepinephrine and/or dopamine do not function as primary neurotransmitters in hypoxia (503). However, monoamines are widely distributed in the brain and as such may be expected to contribute in a complex way. Mice lacking the gene for the dopamine transporter (DAT−/−) show a reduced poikilocapnic HVR (805), but data from these mice are difficult to interpret because both carotid body and central dopamine levels will be increased. The same problem is encountered in those studies using dopaminergic blockers that act both peripherally and centrally. As is also the case for serotonin (see below), the influence of central dopamine on the HVR is not limited to the ventilatory component of the response but also concerns the hypometabolic/hypothermic part of it. For example, infusion of haloperidol in the third cerebral ventricle of rats (aimed at exerting effects in the hypothalamus) reduced the drop in hypoxia-induced body temperature without affecting the ventilatory component (35). Dopamine 1 receptors in the anterventral preoptic region of the hypothalamus in particular seem to be involved in this response (36).

Several regions belonging to the central neuronal respiratory network contain noradrenergic/adrenergic neurons, notably the A2 noradrenergic cell group in the caudal NTS where carotid body afferents terminate, the A5 noradrenergic cell group in the ventrolateral pons, the A6 group in the locus coeruleus in the dorsolateral pons, and the A1/C1 noradrenergic cell groups in the ventrolateral medulla (255, 803). All these regions may contribute to the magnitude and/or shaping of the HVR. For example, Coles et al. (140) reported that lesions in the A5 group reduce or abolish the reduction of respiratory frequency following hypoxia. The A2 group, containing neurons responsive to dopamine, is considered to play a modulatory role in the ventilatory acclimatization to chronic hypoxia; the increase in CNS gain (CNS gain is defined in sect. vF4) in chronic hypoxia may be related to (temporal) upregulation of local tyrosine hydroxylase (references in sect. vD).

In the caudal NTS, norepinephrine can influence both glutamatergic and GABAergic transmission via presynaptic receptors and may thus have variable effects on the HVR (873 and references cited therein). Neurons in the A1 and C1 groups are activated in acute hypoxia but do not seem to have an altered catecholamine turnover in chronic hypoxia (221, 722, 782). The contribution of A6 neurons will be discussed in the subsection below on the role of central NO.

Serotonergic neurons in the brain stem are mainly concentrated in medullary, pontine, and mesencephalic raphe nuclei (references in Ref. 322) and may be of special interest for the HVR for several reasons. First, they have connections with respiratory motoneurons and are able to increase their excitability (322, and references cited therein). Second, they are crucial for homeostatic thermoregulation. About 50% of 5-HT neurons in raphe pallidus/obscurus increase their activity upon environmental and/or hypothalamic cooling, and there is growing evidence that raphe neurons modulate the hypothermic component of the HVR (323, 324). Third, they mediate episodic hypoxia-induced long-term facilitation (LTF) of breathing by causing an enhanced release of 5-HT into the phrenic motor nucleus (230, 481; for a discussion on LTF, see sect. vG; note that LTF can also be evoked by electrical simulation of raphe obscurus neurons, Ref. 512). And finally, many raphe neurons also possess intrinsic CO2 sensitivity (322), so at least in the secondary phase of poikilocapnic hypoxia they will certainly have an influence on ventilatory output.

Activation of neurons in the nucleus raphe magnus attenuated the processing of afferent input from the carotid bodies by the NTS in anesthetized rats (602). Microdialysis of the GABA receptor agonist muscimol into raphe magnus of conscious rats augmented the poikilocapnic HVR measured at an environmental temperature of 30°C (within the thermoneutral zone for rats) but reduced it at 24.5–26.5°C; muscimol reduced body temperature at 24.5–26.5°C and increased the anapyrexic component of the HVR without influencing these parameters at 30°C (772). In the same species, lesions of raphe magnus neurons with ibotenic acid (unspecific for serotonergic neurons) increased the hypoxia-induced rise in ventilation measured at an ambient temperature of 25°C without affecting the anapyrexic response (256). However, microinjection of a specific 5-HT1A receptor antagonist into raphe magnus decreased the poikilocapnic HVR (557). Unless it is speculated that the applied antagonist (WAY-100635) acted on presynaptic inhibitory receptors (autoceptors) on serotonergic neurons, the latter finding seems difficult to reconcile with the stimulatory effects of local lesions and muscimol application on the ventilatory component of the HVR.
To address the role of central serotonin in respiratory and thermoregulatory control, Richerson and co-workers (324) utilized conditional knockout mice almost entirely devoid of central 5-HT neurons, designated as Lmx1b<sup>bp/bp</sup>. These mice are deficient in the capacity to generate heat, resulting in a rapid lowering of body temperature when placed in a cold environment; ventilatory CO<sub>2</sub> sensitivity is also greatly reduced in these animals (324). Although they had the expected temperature-dependent hypoxia-induced changes in ventilation and metabolism (i.e., lowering the environmental temperature decreased the ventilatory component of the HVR but increased the anapyrexic response), these animals had a reduced ventilatory and V<sub>E</sub>/V<sub>O₂</sub> response compared with wild types when the HVR was measured at 25°C. At an ambient temperature of 30°C, no difference in HVR between both genotypes was visible, whilst intracerebroventricular administration of serotonin restored the impaired hypoxic response to CO<sub>2</sub> but did not alter the (normal) HVR (323). Whether Lmx1b<sup>bp/bp</sup> mice would fail to show episodic hypoxia-induced LTF of breathing (see sect. v) remains to be examined. Hodges and co-workers (323, 324) suggested that serotonergic neurons are not directly involved in the HVR per se but may be critically important to thermogenesis.

Mice showing overwhelming amounts of serotonin in brain extracellular fluid are represented by a genotype lacking expression of the serotonin transporter (5-HTT). 5-HTT knockout mice showed a reduced activity of brain serotonergic neurons and decreased 5-HT<sub>1A</sub> receptor binding (444). At room temperature, these animals had a larger ventilation and higher metabolism but normal body temperature. When challenged with 10% O<sub>2</sub>, they responded with a greater increase in ventilation and larger fall in metabolism than wild types, but the change in the V<sub>E</sub>/V<sub>O₂</sub> ratio was equal in both genotypes (444).

A role of serotonergic neurons in medullary raphe in thermoregulation is consistent with the finding that inhibition of raphe pallidus neurons (by means of local injection of the GABA antagonist bicuculline) increased brown adipose sympathetic nerve activity, responsible for thermogenesis in cold stress (482). In the rat, an increased activity of brown adipose sympathetic nerve activity (thought to underlie the anapyrexic response to hypoxia) can be induced in several ways: cold stress (i.e., skin cooling), activation of neurons in preoptic thalamic regions, and disinhibition of raphe pallidus neurons and also of neurons in the commissural subnucleus of the NTS (482). Interestingly, carotid body activation with NaCN or hypoxia completely inhibits the increase in sympathetic outflow to brown adipose tissue by these interventions. This suggests that the carotid body not only plays a pivotal role in the hypoxia-induced rise in ventilation but also in the anapyrexic response (further references in Ref. 482).

From all of the above data, it is not easy to distill a clear and detailed picture of the role of central serotonergic neurons in the HVR. A few general conclusions, however, seem justified. First, in rodents, serotonergic neurons in medullary raphe are crucial for the homeostatic control of body temperature and participate in both the ventilatory and hypometabolic/anapyretic components of the HVR. Second, serotonergic neurons in raphe nuclei possessing connections with respiratory (premotor) neurons are activated in hypoxia. Third, activation of serotonergic neurons in raphe magnus tend to dampen the rise in ventilation and to augment the fall in body temperature, but the magnitude of these effects depends on the ambient temperature. And, finally, the role of raphe serotonergic neurons in hypoxia-induced LTF of breathing is well established (discussed in sect. vG1).

C) Central NO and HVR. I) NTS. Carotid body stimulation in hypoxia is followed by release of glutamate and production of NO in the caudal (commissural) NTS. When injected in the NTS, the NO donor sodium nitroprusside augments the HVR while the nonselective NOS inhibitor N<sup>ω</sup>-monomethyl-arginine (L-NMMA) reduces it. These observations in unanesthetized rats, together with the finding that the increased production of NO is inhibited by NMDA receptor antagonism, led to the suggestion that NO and glutamate operate in the NTS in a positive feedback cycle to increase the HVR (562; see also Ref. 257). Glutamate augments the HVR by activating NMDA receptors in projection areas of carotid body afferents in the NTS that are rich in NO (130, 286, 288, 446; reviewed in Ref. 287). In the conscious rat, injection of both the nonselective NOS inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) and the selective neuronal inhibitor of the enzyme N<sup>ω</sup>-propyl-L-arginine in the caudal NTS reduced the increase in respiratory frequency induced by carotid body stimulation with KCN (291).

Lipton et al. (451) proposed an involvement of NO in the HVR via endogenous production of S-nitrothiosols within the NTS. Hemoglobin desaturation goes along with formation of S-nitrothiosols from glutathione and cysteine and Hb-bound NO producing S-nitroso-glutathione (GSNO) and S-nitroso-L-cysteine (L-CSNO), respectively. GSNO is converted into CGSNO (S-nitroso-cysteinyglutamine), and this requires the enzyme γ-glutamylpeptidase (γ-GT; Ref. 451 and references therein). Local injection of all three nitrothiosols into the NTS mimicked the HVR in conscious rats including its recovery phase (short-term potentiation) after removal of the hypoxic stimulus, and the same was observed after injection of plasma from deoxygenated but not oxygenated blood (451). The effect of CGSNO was inhibited by pretreatment with a blocker of γ-GT, suggesting that conversion of GSNO into CGSNO is an essential step in producing the effect of the former. L-CSNO is less stable in vivo, and thus Lipton et al. (451) hypothesized that γ-GT would be required for a normal HVR to occur and found the short-term potentiation to be abolished in γ-GT-deficient mice. Apparently, however,
the hypoxia-induced rise in ventilation was not eliminated in γ-GT−/− mice, but their report did not show the magnitude of this initial increase (451). From the fact that in the rat denervation of the carotid bodies reduces the HVR with at least 70% and virtually eliminates it in other species, the quantitative contribution of S-nitrothiosols can be estimated to be small or negligible unless carotid body-activated NOS within the NTS would play a major role in producing these molecules during hypoxia. On the other hand, the reported increase of the HVR in humans by N-acetylcysteine (321) could be related to generation of S-nitrothiosols with the aid of Hb-bound NO (578), rather than to the antioxidant properties of this molecule.

II) Pons and locus coeruleus. In the pontine reticular formation, NO promotes the release of acetylcholine which induces sleep associated with rapid eye movements and respiratory depression (442). The dorsolateral pons uses NO to promote the termination of inspiration during lung inflation (pneumotaxic mechanism). In awake rats, electrolytic bilateral lesions in the locus coeruleus substantially augmented the HVR (224). In addition, whereas acute intracerebroventricular administration of the nonselective NOS inhibitor L-NAME almost completely inhibited the HVR in control animals, it did so by only ∼50% in those with the bilateral lesions, suggesting a substantial contribution of the locus coeruleus to the inhibition in intact animals (224). The locus coeruleus contains the A6 cell group that is considered the major noradrenergic cell group in the brain stem (references in Ref. 803). Microinjection of L-NAME in the locus coeruleus had an impressive inhibitory effect on the HVR, indicating that the “permissive” effect of NO may be due to inhibition of, presumably noradrenergic, neurons in this area (225). It is unclear why in another study in rats chronic injection (7 days) of small amounts of L-NAME into the right cerebral ventricle did not modify the HVR unless it is hypothesized that chronic NO shortage may be compensated by upregulation of NOS (688). Notwithstanding this latter study, all data taken together clearly identify the locus coeruleus as an important inhibitory modulator of the HVR, possibly analogous to an active inhibitory role of lateral pontine regions in the hypoxic inhibition of breathing in newborn lambs (522; see also sect. ii).

III) Other regions. Microinjection of the nonselective NOS inhibitor L-NMMA into the RVLM reduced the HVR in rats but not the hypothemic response (173). The same inhibitor had a similar effect when injected into the nucleus raphe magnus (556). A possible role of brain stem NO in the acclimatization to chronic hypoxia has been briefly mentioned in section Vf.

In humans, the acute ventilatory response to hypoxia does not seem to be influenced by systemic administration of L-NMMA (347). The intravenous dose applied in this study (∼62 μg/kg), however, was much smaller than that mostly used for NOS inhibitors in animal studies (including those applying ventricular administrations), so perhaps inhibitor concentrations at target sites within the brain were too small to produce significant effects (although blood pressure clearly increased; Ref. 347).

D. Hypoxic Ventilatory Decline in the Adult Mammal

In adult mammals, the ventilatory response to sustained (15–30 min) hypoxia (end-tidal PO2 50–55 Torr and SaO2 ~80%) is biphasic (Figs. 5 and 6; Refs. 162, 204, 370, 383, 464, 752, 806). During moderate isocapnic hypoxia (i.e., arterial PO2 ∼50 Torr) lasting >3–5 min, ventilation shows a slow decline from its peak (75–150% above normoxic resting values) to reach a new steady-state level (25–40% of normoxic values) within 15–20 min (162, 204, 236, 383). The slow decline was formerly known as “hypoxic ventilatory depression”; current names include hypoxic ventilatory decline (HVD), hypoxic ventilatory roll-off, and secondary roll-off of the HVR.

Although consistently found across mammalian species, dogs and goats are the exception with little or no HVD during exposure to moderate hypoxia (113, 151, 546). However, HVD is dependent on various factors, such as age, sex, CNS arousal state, (type of) anesthesia, breathing route, and hypoxic test (12, 111, 498, 677, 773). For example, HVD is most pronounced under isocapnic experimental conditions, while the poikilocapnic HVD is much smaller in magnitude (12).

In the awake state, the ventilatory response to sustained isocapnic hypoxia has specific characteristics. 1) The magnitude of HVD is proportionally related to the size of the initial hyperventilatory response to acute hypoxia (162, 263, 266, 383). 2) Bilateral carotid body resection or chemodenervation results in the loss of the V1 (inspired ventilation) response to acute hypoxia and absence of HVD (Fig. 5; Refs. 383, 464). 3) HVD persists beyond the initial period of hypoxic exposure. The V1 response to acute hypoxia following 20 min of moderate isocapnic hypoxia and 5 min of air breathing is depressed by >50%. This delayed recovery persists for up to 1 h (162, 205). 4) Suppression of the peripheral drive with low-dose dopamine yields no HVD during 20-min hypoxia. And, despite the presence of initial central hypoxia, a subsequent V1 response to acute hypoxia develops fully (Fig. 6; Ref. 162). 5) The magnitude of the fall in ventilation upon the relief of hypoxia is smaller than the V1 response generated at the onset of hypoxia (204, 382). 6) Awake humans and awake cats display similar response characteristics with respect to characteristics 1–3 and 5 (Fig. 5; Refs. 204, 464).

These observations (most importantly characteristic 4) suggest that in conscious mammals the peripheral chemoreceptors play a pivotal role in the development of HVD. The issue remains whether HVD is due to adapta-
The hypothesis that HVD is related to peripheral chemoreceptor adaptation is supported by characteristics 1, 2, and 5 and is further corroborated by the observation that hypoxic sensitivity declines during the development of HVD (37). However, in the anesthetized cat, the peripheral chemoreceptors do not adapt during hypoxic exposure in terms of ventilation and afferent nerve activity, indicating that an exclusive peripheral mechanism is unlikely (17, 58, 807). Furthermore, characteristics 3 and 4 indicate an effect of HVD on HVR beyond hypoxic exposure when peripheral chemoreceptor activity in terms of the $V_t$ response to CO$_2$ has normalized (57, 267, 461). The current hypothesis is that, at least in the awake mammal, a large part of HVD is due to modulation of glutamatergic excitatory activity within the NTS (arising from afferent carotid body input) shifting the excitatory/inhibitory balance towards a net inhibitory effect on $V_t$ (162, 266, 810).

At the termination of hypoxia, changes in neurotransmitter turnover are not immediate and, therefore, the depressant effects of hypoxia wane slowly. In the awake state, a small part of HVD is related to an increase in brain blood flow and the resultant washout of acid metabolites/H$^+$ from the brain. Suzuki et al. (746) showed a decrease in the gradient between jugular venous and arterial PCO$_2$ of $\sim$2 Torr during 15 min of moderate isocapnic hypoxia. Assuming that jugular venous PCO$_2$ approximates brain tissue PCO$_2$, $\sim$10–20% of measured HVD is related to an increase in brain blood flow in the adult awake human. Finally, at deeper levels of hypoxia, substrate insufficiency and depressed cellular metabolism may become the major origin of HVD (435, 540).

Various mechanisms could explain the decrease in excitatory neuronal output during sustained hypoxia. For example, as suggested by Kazemi and Hoop (379), peripheral chemoreceptor afferents cause the enhancement of...
Glutamatergic excitation in areas of the brain stem involved in hypoxic ventilatory control. Glutamate serves as the precursor of GABA, and conversion of glutamate into GABA may enhance GABAergic activity with consequently a net inhibitory effect on $V^\prime$. In the awake rat, Tabata et al. (752) measured an increased GABA concentration in the extracellular fluid in the NTS during HVD development, which was absent in animals after bilateral carotid body resection. Both the reduction in $O_2$ availability and intracellular acidosis increase GABA levels in the brain with kinetics that are fast enough to account for HVD (increased synthesis from glutamate through the brain with kinetics that are fast enough to account for $V^\prime$, 823). Comparable observations are made in carotid body perfusion experiments and anesthetized animals (289). Via this pathway, the NTS itself could be the source of increased GABA during hypoxia (540, 752). However, other sources of GABA are also possible because the NTS contains many terminals immunoreactive to GABA that may inhibit glutamatergic inputs (e.g., Ref. 673 and references therein). GABAergic neurons in the NTS send inhibitory efferents to the caudal ventrolateral medulla and also to the locus coeruleus, providing other possible pathways involved in HVD (7, 795). Other regions such as the hypothalamus may also participate in HVD via a GABAergic mechanism. For example, Lemus et al. (440) depicted an interesting circuitry participating in the glycemic response of carotid body stimulation involving antidiuretic hormone (ADH)-secreting neurons in the supraoptic nucleus and ADH receptors on the membrane of GABAergic neurons in the NTS that may originate from the paraventricular nucleus (PVN) of the hypothalamus. It is known that arginine vasopressin-containing neurons in the PVN and supraoptic nucleus are stimulated by hypoxia (709 and references therein), but it remains to be seen whether NTS neurons could be directly inhibited during hypoxia via this pathway.

Other inhibitory modulators possibly involved in HVD include dopamine, adenosine, serotonin, endogenous opioid peptides, lactic acid, NO, and $Ca^{2+}$ (Tables 1 and 2 for references). A relatively new observation is that of Gozal et al. (289) who examined the role of the platelet-derived growth factor (PDGF) isoforms PDGF-AA (with two A chains and acting on the PDGF-α receptor) and PDGF-BB (with two B chains and acting on the PDGF-β receptor) in the NTS during hypoxia. The NTS and other brain stem respiratory nuclei show an abundant expression of the PDGF-β but not PDGF-α receptor. In contrast to PDGF-AA, the isoform containing two B chains and its receptor PDGF-β appeared to play a functional role in the development of HVD. Awake mice with a heterozygous mutation of the PDGF-β receptor had an intact $V^\prime$ response to acute hypoxia but a diminished HVD, an effect which was unaffected by the injection of a PDGF-β receptor antagonist. However, wild-type animals showed a reduced HVD after the antagonist. Many NTS neurons in wild-type mice (and in rats) showed increased expression of PDGF-B chain mRNA and protein (release) in hypoxia. These data suggest an inhibitory role for PDGF-BB and its receptor on ventilation during sustained hypoxia, possibly by inhibiting NMDA receptors within the NTS (289).

There is strong evidence that HVD development during anesthesia is uncoupled from peripheral chemoreceptor activity (657). For example, in anesthetized cats subjected to artificial brain stem perfusion, central hypoxia with the carotid bodies kept normoxic results in a decline in ventilation with dynamics similar to that observed after central changes in $P_{CO_2}$ (823). Comparable observations are made in carotid body perfusion experiments and anesthetized chemodenervated animals (361, 538, 543; Table 2). Similarly, in humans exposed to halothane, the initial hyperventilatory response is greatly reduced while the absolute magnitude of the HVD is similar to that observed in the awake state (160). Furthermore, the ventilatory response to a step out of sustained hypoxia (the off-response) shows a marked undershoot and is similar in magnitude to the initial hyperventilatory response (the on-response). These symmetric on- and off-responses in humans exposed to halothane indicate that in this condition peripheral sensitivity, although reduced under of low-dose halothane, remains unchanged during HVD. Similar observations were made in humans injected with the intravenous anesthetic propofol (549). These data sharply contrast with data in awake humans and animals that do not show HVD development after carotid body resection or when the peripheral drive is reduced by dopamine (Fig. 6; Refs. 162, 383, 464).

Fig. 6. Dopamine and hypoxic ventilatory decline (HVD) in humans. Ventilatory response to 20-min isocapnic hypoxia ($SpO_2 = 80\%$), 5-min normoxia and subsequently a second hypoxic episode ($SpO_2 = 80\%$, lasting 5 min). Closed circles, control response showing the HVD from early to late hypoxia and a marked reduced response to a second hypoxic exposure; open circles, low-dose dopamine infusion (gray bar: $3 \mu g \cdot kg^{-1} \cdot min^{-1}$) during sustained hypoxia blunts the initial hyperventilatory response and prevents development of HVD. The ventilatory response to the second hypoxic exposure develops fully. Data are the means of 16 subjects. [Adapted from Dahan et al. (102).]
During anesthesia, when the peripheral drive is either absent or severely reduced, it seems appropriate to attribute the development of HVD to an effect arising selectively from within the CNS. One possibility is that while CBF-induced HVD plays only a minor role in the awake state, it becomes the major contributor during anesthesia. In the anesthetized cat, brain blood flow-related HVD does explain the similarities in ventilatory time constants of central CO₂ steps and hypoxic steps, respectively, restricted to the brain stem (823). It would also explain the

**TABLE 1. Effect of pharmacological interventions on the development of HVD in adult humans**

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Arousal State</th>
<th>Effect</th>
<th>Comments</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₂-Adrenergic receptor</td>
<td>Clonidine</td>
<td>Sedated</td>
<td>AHR= HVD=</td>
<td>In line with the observation that the magnitude of HVD is related to AHR</td>
<td>236</td>
</tr>
<tr>
<td>Adenosine receptor</td>
<td>Aminophylline (adenosine antagonist)</td>
<td>Awake</td>
<td>AHR= HVD ↓</td>
<td>Data suggest a role for adenosine in the development of HVD</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>Dipryridamole (adenosine reuptake inhibitor)</td>
<td>Awake</td>
<td>AHR ↑ HVD ↑</td>
<td>Pretreatment with aminophylline abolished the dipryridamole effect; data indicate a role for adenosine in HVD development</td>
<td>859</td>
</tr>
<tr>
<td>Calcium channel</td>
<td>Verapamil (Ca²⁺ channel blocker)</td>
<td>Awake</td>
<td>AHR= HVD=</td>
<td>Data suggest no involvement of calcium ions in HVD development</td>
<td>459</td>
</tr>
<tr>
<td>D₂ receptor</td>
<td>Low-dose dopamine</td>
<td>Awake</td>
<td>AHR ↓ HVD ↓</td>
<td>Dopamine acts peripherally at this dose; the reduced HVD prevents depression of a subsequent Vᵢ response to acute hypoxia</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Haloperidol (central and peripheral D₂ antagonist)</td>
<td>Awake</td>
<td>AHR ↓ HVD=</td>
<td>Effect contrasts findings in awake cats (see Refs. 769 and 460)</td>
<td>38, 821, 589</td>
</tr>
<tr>
<td></td>
<td>Domperidone (peripheral D₂ antagonist)</td>
<td>Awake</td>
<td>AHR ↑ HVD=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Dichloroacetate (blocks lactate production)</td>
<td>Awake</td>
<td>AHR= HVD=</td>
<td>Data suggest that brain lactic acidosis is unlikely involved in HVD development</td>
<td>264</td>
</tr>
<tr>
<td>Opioid receptor</td>
<td>Naloxone (opioid receptor antagonist)</td>
<td>Awake</td>
<td>AHR= HVD=</td>
<td>Data suggest no involvement of endogenous opioid system in HVD development</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>Alfentanil (μ-opioid receptor agonist)</td>
<td>Awake</td>
<td>AHR ↓ HVD=</td>
<td>Data suggest that exogenous opioids potentiate HVD</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Morphine (μ-opioid receptor agonist)</td>
<td>Awake</td>
<td>AHR ↓ HVD=</td>
<td></td>
<td>680</td>
</tr>
<tr>
<td></td>
<td>Methysergide (5-HT receptor antagonist)</td>
<td>Awake</td>
<td>AHR ▼ HVD=</td>
<td></td>
<td>25</td>
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<td></td>
<td>Somatostatin</td>
<td>Awake</td>
<td>AHR ▼ HVD=</td>
<td>Effect possibly via disinhibition of GABAergic inhibitory system</td>
<td>231</td>
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<td></td>
<td>Carotid body</td>
<td>Awake</td>
<td>AHR ↑ HVD ↑</td>
<td>In line with the observation that the magnitude of HVD is related to AHR</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Intrathecal morphine</td>
<td>Awake</td>
<td>AHR ▼ HVD=</td>
<td></td>
<td>298</td>
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<tr>
<td></td>
<td>Intrathalamic hypnotics</td>
<td>Lightly anesthetized</td>
<td>AHR ▼ HVD=</td>
<td>Data suggest that the link between AHR and HVD is lost during anesthesia</td>
<td>798</td>
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<tr>
<td></td>
<td>Low-dose inhalational anesthetics (halothane, isoflurane, enfurane)</td>
<td>Lightly anesthetized</td>
<td>AHR ▼ HVD=</td>
<td></td>
<td>535, 235, 549</td>
</tr>
<tr>
<td></td>
<td>Propofol (intravenous anesthetic)</td>
<td>Lightly anesthetized</td>
<td>AHR ▼ HVD=</td>
<td>Propofol may have increased HVD via its action at the GABA receptor complex; propofol had no effect on intermittent hypoxic ventilation</td>
<td></td>
</tr>
</tbody>
</table>

AHR, acute hypoxic ventilatory response; HVD, hypoxic ventilatory decline; Vᵢ, inspired ventilation.
<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Animal</th>
<th>Arousal State</th>
<th>Effect</th>
<th>Comments</th>
<th>Reference Nos.</th>
</tr>
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<tbody>
<tr>
<td>Adenosine receptor</td>
<td>Aminophylline (adenosine antagonist)</td>
<td>Cat</td>
<td>Anesthetized (chloralose-urethane)</td>
<td>HVD ↓</td>
<td>The carotid sinus nerves and vagi of the animals were bilaterally denervated</td>
<td>361</td>
</tr>
<tr>
<td></td>
<td>Theophylline iv and icv (adenosine antagonist)</td>
<td>Cat</td>
<td>Anesthetized (sodium pentothal)</td>
<td>iv: AHR ↑</td>
<td>Data from these interventions suggest involvement of adenosine in development of HVD</td>
<td>866</td>
</tr>
<tr>
<td>D₂ receptor</td>
<td>Haloperidol (central and peripheral D₂ antagonist)</td>
<td>Cat</td>
<td>Awake</td>
<td>AHR= HVD ↓</td>
<td>HVD was abolished by haloperidol</td>
<td>769</td>
</tr>
<tr>
<td></td>
<td>Domperidone (peripheral D₂ antagonist)</td>
<td>Cat</td>
<td>Awake</td>
<td>AHR= HVD=</td>
<td>Identical results as in the awake cat; carotid sinus nerve activity remained constant during HVD development</td>
<td>769</td>
</tr>
<tr>
<td>GABA receptor</td>
<td>Bicuculline (GABA antagonist) microinjected in the NTS</td>
<td>Rat</td>
<td>Awake</td>
<td>HVD ↓</td>
<td>GABA antagonists reduced HVD in intact animals only; in carotid body denervated animals, no HVD was present and no HVD developed upon injection of GABA antagonists during hypoxia</td>
<td>752</td>
</tr>
<tr>
<td></td>
<td>Bicuculline iv (GABA antagonist)</td>
<td>Cat</td>
<td>Anesthetized (chloralose-urethane)</td>
<td>HVD ↓</td>
<td>Animals were glomectomized; phrenic nerve response to progressive CO was measured</td>
<td>508</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Dichloroacetate (prevents lactate production)</td>
<td>Cat</td>
<td>Anesthetized (chloralose-urethane)</td>
<td>HVD ↓</td>
<td>Animals were peripherally chemodenervated; progressive hypoxia induced by inhalation of CO; data suggest a role for lactic acid in HVD generation</td>
<td>543</td>
</tr>
<tr>
<td>Nitric oxide synthase (NOS)</td>
<td>N⁵- nitro-L-Arginine (a nonspecific NOS blocker)</td>
<td>Rat</td>
<td>Awake</td>
<td>HVD ↑</td>
<td>Control animals did not display HVD</td>
<td>290</td>
</tr>
<tr>
<td>Opioid receptor</td>
<td>Naloxone (opioid receptor antagonist)</td>
<td>Cat</td>
<td>Anesthetized (chloralose-urethane)</td>
<td>HVD ↓</td>
<td>Animals were peripherally chemodenervated; ventilatory response to progressive CO was measured</td>
<td>541</td>
</tr>
<tr>
<td></td>
<td>Morphine (µ-opioid receptor agonist)</td>
<td>Cat</td>
<td>Anesthetized (chloralose-urethane)</td>
<td>HVD ↓</td>
<td>Steady-state response to sustained hypoxia remained unaltered by morphine, suggesting a reduced HVD possibly by reducing brain blood flow response to hypoxia</td>
<td>61</td>
</tr>
</tbody>
</table>
symmetry in on- and off-responses observed in halothane-exposed humans. Manipulating brain blood flow either hemodynamically (for example, by aortic balloon inflation), pharmacologically (for example, by infusion of papaverine to blood perfusing the brain stem), or by exposure to central hypoxia produce a similar decrease in $V_{\dot{I}}$ that is consistent with washout of central $CO_2$ (60, 293, 542, 823). Finally, we cannot exclude an effect of modulators accumulating during hypoxia in the anesthetized mammal. Indeed, some enhancement of GABAergic inhibition of $V_{\dot{I}}$ during hypoxia is expected, especially from anesthetics that have agonistic activity at the GABA receptor. However, at $PO_2$ values /H11022 50 Torr, these effects are probably small with regard to the reduced tissue $PCO_2$ by changes in BBF (794).

IV. GENETIC INFLUENCE ON THE VENTILATORY RESPONSE TO HYPOXIA

A. The Genome and HVR

The genotype plays an important role in the magnitude of the HVR and consequently explains part of the large intersubject response variability (734). Measurements of the HVR in humans show that normal healthy volunteers may vary a factor of 20 in the magnitude of their response. This range is much larger than expected from just normal physiological variability and technical, environmental, and/or emotional factors. Furthermore, data from high-altitude residents indicate that genotype may not only influence the immediate response to hypoxia but possibly also the ventilatory response to sustained hypoxia and ventilatory adaptation to high altitude (519).

1. Human studies

In humans, evidence for a genetic effect on HVR comes primarily from family and population studies. First-degree healthy family members of patients with pulmonary disease associated with hypoventilation and $CO_2$ retention and nonathletic parents and siblings of endurance athletes show a familial clustering of HVR magnitudes at the low end of the spectrum (see Ref. 832 and references cited therein). Similar observations were made in families with a history of SIDS and obstructive sleep apnea (734). Twin studies show greater similarities in the magnitude of HVR among homozygotic twins compared with heterozygotic twins (see Ref. 734 and references cited therein). Most but not all interpopulation comparisons indicate the existence of heritable HVR phenotypes linked to specific populations (96, 97, 152, 783). Strong evidence for a hereditary component comes from studies in Asians and South Americans. Children from Tibetan
and Han (Chinese) parents tend to be in the range observed in the Han population (152). With the use of ancestry-informative genetic markers, an evolutionary origin was found for the low HVR in Andean high-altitude natives of Quechua origin (97).

2. Animal studies

There is ample proof that the divergence in ventilatory behavior between different inbred strains of the same species reflects genetic influences more than effects from body mass, age, or sex. This also applies to the ventilatory response to moderate hypoxia and the survival response to severe hypoxia (874). In rats, most studies show an effect of strain on the magnitude of HVR (50, 274, 736, 739, 834). Some strains are difficult to compare as underlying disease may affect carotid body function. For example, New Zealand hypertensive rats suffer from sclerotic damage to the carotid artery and its branches (50). Strains may differ in the magnitude of the HVR by up to a factor of seven (834). The differences are not only related to the carotid body response to hypoxia but also to central neural processing during and immediately after exposure to short episodes of hypoxia (274, 739). As stated by Golder et al. (274), the choice of rat strain can (critically) influence conclusions concerning the control of breathing. This is also true for drug studies. For example, in rat, the peripheral dopamine D2 receptor antagonist domperidone and the NOS inhibitor l-NAME differentially affect ventilation and the HVR depending on the genetic background (737, 738).

To pinpoint the HVR phenotype to a specific rat chromosome, Forster et al. (240) used the technique of chromosomal substitution in which an entire chromosome from one inbred rat strain is introgressed into the genetic background of another to create a consomic rat. Initially, five chromosomes were studied. HVR was not determined by any of the genes on these chromosomes, although genes on chromosomes 9 and 18 did contribute to HVD and/or a reduced metabolic rate during hypoxia. The results of a second study indicate that there are complex gene-gender interactions on HVR (199).

Like rats, inbred mice strains vary significantly in the magnitude of their HVR. Tankersley et al. (760) examined the HVR in eight inbred strains. The DBA/2J strain had the greatest HVR, while the A/J strain displayed the lowest response. Tankersley et al. (760) did not take the brain state of the animals into account, and in this this regard, it is interesting that A/J mice are more prone to sleep than DBA/2J mice and have a higher arousal threshold in hypoxia (669). So possibly the ventilatory response could be the reflection of a stress response. However, the difference in frequency of hypoxic events during sleep (larger in the A/J strain) was inversely related to the hypoxic ventilatory sensitivity, whilst the arousal threshold upon tactile/auditory stimuli was not different between both strains (669). The difference in HVR was associated with structural and functional changes in the carotid bodies (858). Compared with the A/J strain, the carotid bodies of the DBA/2J strain were larger, and acetylcholine increased intracellular Ca2+ in more cells. These morphological and functional differences are accompanied by differences in gene expression (30). There were differences in expression of genes related to neurotransmitter metabolism, synaptic vesicles, and neural crest cell migration/development with less expression in A/J mice. An important difference was the reduced expression of the β-subunit of maxi-K channels in A/J mice. Further studies from Tankersley and co-workers (758, 759, 761) aimed to uncover the genes involved in the HVR phenotype. Data from an intercross study between C5H/3J and C57BL/6J mice suggest that HVR in terms of respiratory timing is dependent on only a handful of genes (759). A putative quantitative trait locus on chromosome 9 could be identified with a significant association with several hypoxic ventilatory variables (758), while another locus on chromosome 3 was significantly associated with hypoxic inspiratory drive. Both loci explained 26–30% of the phenotypic variation among F2 offspring. The interaction between hypoxia and hypercapnia on breathing was linked to two different loci on chromosomes 1 and 5 (761). The simplicity of this genetic model is appealing and is much less complex than in the rat. It is doubtful whether such a restricted model also applies to the human HVR phenotype.

B. Mutations and HVR

Examples of targeted gene deletions in mice to study the process of oxygen sensing at the carotid bodies and/or to understand the peripheral and central processing of the carotid body response to hypoxia are discussed in section III. Here we briefly discuss mouse strains developed to study specific diseases and that show altered ventilatory responses to hypoxia. We further discuss two human conditions in which one specific mutation is linked to an altered HVR.

Various mouse strains have been engineered to study the Congenital Central Hypoventilation Syndrome (CCHS), some of which have recently been reviewed (see Ref. 259). Particularly genes of the endothelin and c-ret pathway have been the focus of interest, for example, endothelin converting enzyme (ECE1), endothelin-1 (EDN1), endothelin-3 (EDN3), endothelin receptor A (Ednra), endothelin receptor B (Ednrb), Mash-1, c-ret, Phox2a, Phox2b, brain-derived neurotrophic factor (Bdnf), and orphan homeobox (Rnx) genes (169, 259, 849). Knockouts at these genes display a variable respiratory phenotype with reduced hypercapnic response and/or HVR in ECE1+/−, EDN1+/−, Ednra−/− and Mash-1+/− newborn mice; however, with the
exception of $EDN1^{-/-}$ mice, these genotypes did not display abnormalities as adults. This is an important limitation for a disease that in humans persists throughout life (259). There are other mice models associated with CHHS such as the Nurr1 mutant mouse and the microphthalmia-associated transcription factor (Mitf) mutant mouse (555, 754). Nurr1 mutant mice show complete agenesis of midbrain dopamine cells. Newborn Nurr1 knockout mice have a severely disturbed pattern of breathing with a blunted HVR (555). Mitf plays an essential role in the differentiation of neural crest-derived melanocytes. Mitf mutant mice display an increased HVR. The authors relate this to a reduction of melanocyte-derived opioids within the CNS (754). This suggests an inhibitory role of endogenous opioids on the central ventilatory network and is in agreement with observations in $\mu$-opioid receptor gene knockout mice (158).

A mouse model associated with the Rett syndrome is represented by a genotype with a ubiquitous deletion of one allele of the X-linked $Mecp2$ gene, encoding the DNA-binding protein methyl CpG ($Mecp2$) involved in gene silencing. Female $Mecp2^{+/+}$ mice have a respiratory phenotype resembling that of the Rett syndrome and that is characterized by episodes of hyperventilation and respiratory depression (75). The (isocapnic) HVR in heterozygous females is larger than in wild types; mice with a selective deletion of the protein in neurons do also display an enhanced HVR but do not show the typical episodes of respiratory depression (75). It is interesting to note that different respiratory phenotypes also exist between different mouse strains with the gene deletion (references in Ref. 563).

In humans, carotid body tumors are rare and often associated with chronic hypoxia from altitude, cyanotic heart disease, and pulmonary disease. One hereditary form of carotid body tumor (as part of the hereditary paranganglioma syndrome) is due to a missense mutation within the gene that encodes succinate dehydrogenase D (SDHD; Refs. 48, 154, 213). SDHD is an essential enzyme of the tricarboxylic acid cycle and part of cytochrome $b_{588}$ of the mitochondrial respiratory chain complex II. All patients are heterozygous: animal studies indicate that homozygotes do not survive and die in utero (607). We measured the isocapnic HVR and hypercapnic ventilatory responses in normoxia and hypoxia in 12 tumor-free carriers of the mutation (as tested by MRI; author’s unpublished observation). There were three main observations: 1) hypercapnic ventilatory responses were not different from age-matched healthy controls, 2) the magnitude of the isocapnic HVR was at the low end of “normal” (mean value 0.3 versus 1.2 l.min$^{-1}$.%$^{-1}$ in controls), and 3) in contrast to controls, none of the tested carriers of the SDHD gene showed any $O_2$-$CO_2$ interaction on the HCVR. These data may link the $SDHD$ gene to carotid body oxygen sensing and/or to the well-described multiplicative action of $CO_2$ on the HVR (see sect. vD).

Robbins and co-workers (712) studied the effect of a mutation in the von Hippel-Lindau tumor suppressor protein (VHL) gene. A homozygous missense mutation that impairs but does not ablate VHL function causes Chuvash polycythemia (47, 213). The disease is endemic to the Chuvash population of Russia. In patients with Chuvash polycythemia, binding of VHL to HIF-1$\alpha$ is diminished and hence normal degradation reduced causing the accumulation of HIF (in normoxia) and consequent upregulation of certain target genes such as the erythropoietin gene. The three patients studied showed several abnormalities in respiratory and pulmonary vascular regulation. Most importantly, the isocapnic HVR and pulmonary artery pressure responses to hypoxia were increased compared with healthy and polycythemia control subjects (712). In other words, cardiorespiratory responses to hypoxia were upregulated in patients with increased HIF levels and upregulated HIF target genes. These data are in agreement with animal studies showing downregulated cardiopulmonary responses in HIF-1$\alpha^{-/-}$ mice and attenuated adaptive responses to chronic intermittent hypoxia (see sect. v).

In conclusion, the above examples of the influence of single gene defects (and their inheritance) on the HVR are clear illustrations of a genetic influence. Strain differences in animals and differences in HVR between lowlanders and high-altitude natives illustrate the influence of genomic factors, i.e., the interaction between different genes on the one hand and that between genes and the environment on the other hand. Both respiratory and nonrespiratory stimuli are able to affect the development of the HVR (developmental plasticity) causing alterations in response that may persist throughout adulthood. In section ii we discussed the influence of changes in oxygen tension in the neonatal period. One clear example of a nonrespiratory stimulus leading to plastic changes is stress from neonatal maternal separation that leads to a permanently augmented HVR of male but not female rats (262). Brain stem areas involved in ventilatory control of the neonate have strong plastic capacities depending on specific environmental stimuli and genetic background (735).

V. PATHOPHYSIOLOGICAL INFLUENCES ON THE HYPOXIC VENTILATORY RESPONSE

A. Biological Variability

Apart from a between-subject variability, one has to consider the appreciable within-subject variation in the magnitude of the HVR. The coefficient of variation of repeated HVRs obtained over a 2-h period ranges from 10 to 60% among subjects (mean 20%; Ref. 674). Between-day variability is 20–50% greater than within-day variability. The cause of the large between-subject variability may lie in differences in genetic make-up and race of the different
subjects tested. However, this explains just part of the variability (see sect. iv). Other causes are related to physiological factors (such as age, metabolism, body temperature, the circadian rhythm, hormonal status, and pregnancy), psychological factors (anxiety, full bladder), pathophysiological factors, the use of pharmacological agents that may affect HVR, and environmental factors (previous exposure to hypoxia, altitude). To reduce within- and between-subject variability, it is important to perform hypoxic experiments in subjects that feel comfortable with the setup, protocol, and laboratory surroundings and have no apparent urges that may influence their responses (e.g., a full bladder). Also the investigator should do everything not to influence the subject (for example, whispering may have a negative influence on the subject).

B. Ageing

Breathing is affected by ageing due to a decline in function at multiple levels of the neuromechanical link between chemosensors, brain stem, and ventilatory pump, causing a reduced ability to maintain blood gas homeostasis (360). Morphometric studies of rat carotid bodies indicate the occurrence of degenerative changes in old age (142, 179, 610). The carotid bodies of aged rats (>23 mo) show an increase in extracellular matrix, a reduction in the number and volume of type I cells, and the presence of necrotic cells (142, 179, 610). In the remaining functional type I cells, mitochondrial volumes and neurosecretory fill-up of vesicles are reduced (610). Carotid body catecholamine content and turnover time is reduced with age, as is the catecholamine response to hypoxic stimulation (142). Conde et al. (142) measured afferent carotid sinus nerve activity during hypoxic and CO₂/acidotic stimulation in young (3 mo) and old (18 mo) rats. While no age effect was observed in the nerve response to CO₂/acidosis, in old animals a >50% reduction was observed in the response to hypoxia. Degenerative changes such as fibrosis and loss of glomus tissue have been observed in the human carotid body (311). In 15–30% of humans in their eighth and ninth decade, there may be signs of chronic carotid glomitis with infiltration of T-cell lymphocytes (311). These data collectively indicate important age-dependent morphological and functional changes of the carotid bodies.

A minority of human studies observed a reduced HVR in older persons (age range 64–79 yr; Refs. 412, 604). However, the majority of studies observed no differences in HVR between young (20–30 yr) and old (60–79 yr) (6, 609, 610, 714), while one study observed an increase in HVR (124). The overall picture that emerges from the human literature is that HVR is marginally affected at old age and hence suggests that carotid body function remains unaltered. This stands in sharp contrast to the morphometric and functional studies in carotid bodies of aged rats (see above). However, it may well be that the human studies were performed at the older end of normal adult function when no functional decline in carotid body function is yet apparent (714). Another possible cause for the difference between morphological appearance of the carotid body and its function may be found in the existence of redundancy in carotid body-mediated processes. A reduced number of type I cells may be able to sustain the functional tasks of the carotid bodies (610). Finally, a failing carotid body function in old age may result in plastic changes in brain stem areas involved in the processing of its afferent output, causing the (partial) restoration of the HVR (610).

C. Gender and Pregnancy

Sex hormones (progesterone and the combination progesterone/estrogen as well as testosterone) have a stimulatory effect on breathing and HVR. Progesterone stimulates breathing via hypothalamic sites through estrogen-dependent receptors (46). The role of progesterone in causing an increase in HVR is twofold; it increases carotid body sensitivity to hypoxia, and in combination with estrogen, it has a central modulatory effect on its afferent output (299). Testosterone increases breathing and the HVR probably through an increase in metabolic rate and central modulatory effects (766, 841).

The majority of human studies could not find a difference in HVR between men and women (157, 362, 364, 418, 677, 680, 681). However, most of the studies involved did not control for the phase of the menstrual cycle or tested women in just one phase (often the follicular phase). The number of animal studies on gender effect on HVR is limited, but all, in contrast to the human data, point toward a greater carotid body response to hypoxia in female animals (366, 530, 765). Only part of these sex differences is related to the acute effects of sex steroids. Castration of the adult animals does lead to a reduced HVR in males and females, but the sex differences persist (767). This is not surprising taking into account the long-term developmental and organizational effects of sex steroids on neuronal systems that occur in prenatal and early postnatal life. In this light, the absence of clear sex differences in the human population seems remarkable.

An effect of the menstrual cycle on the HVR is small and ranges from an increased HVR in the luteal phase by 10–20% compared with the follicular phase (157, 840). Pregnancy is associated with hyperventilation due to an increase in metabolic rate and the stimulatory effects of progesterone (636). Also, the HVR is increased during pregnancy, an effect that is already apparent at 20 wk and reaches a maximum at 36 wk (521). More than 60% of the increase in HVR is related to sex hormones, the rest to the
increase in metabolic rate (521, 636). A more extensive overview of the effect of sex steroids on ventilatory control during development and in adult life has been published recently (52).

D. Influence of CO2 on the HVR: O2-CO2 Interaction

In both humans and animals, there is ample evidence for a multiplicative effect of O2 and CO2 on the HVR (12, 150, 178, 156, 195, 494, 517, 777). It is uncertain if quantitatively this O2-CO2 synergism observed at the ventilation level is entirely due to a multiplicative interaction in the carotid bodies (423; further references in Ref. 178).

However, CO2 does not always act as a multiplier of the HVR. In conscious cats, O2-CO2 synergism does not seem to exist, due to a negative interactive effect on tidal volume (575, 576). When anesthetized, however, this species demonstrates interaction at both carotid body and ventilation levels (234, 423). Although displaying multiplicative interaction in the carotid bodies (601), the anesthetized rat only seems to display a positive synergistic hypocapnic-hypoxic action at the level of ventilation at hypocapnic arterial Pco2 values (145, 613). Earlier studies showed that in other species O2-CO2 interaction may be hypoadditive or at best additive (references in Ref. 145; more recent studies are discussed below).

A modulating effect of CO2 on the HVR could originate from three sources: the carotid bodies, a central-peripheral interaction, and/or central O2-CO2 interaction. While a multiplicative interaction in the carotid bodies is evident, the brief overview below shows that there is no consensus as to the modality of the peripheral-central interaction: hyperadditive, simply additive, or hypoadditive. There is no evidence for central O2-CO2 interaction.

1. Carotid sinus nerve recording: properties of type I cells

A relatively simple method to study O2-CO2 interaction in the carotid bodies is with carotid sinus nerve recording. Anesthetized cats, rats, lambs, and rabbits demonstrate O2-CO2 interaction in the carotid sinus nerve (116, 118, 119, 234, 333, 386, 423, 601, 660; further references in Ref. 233) that most likely can be reduced to the properties of type I cells. The few studies that have been performed in type I cells may indeed suggest an interaction between acid/high CO2 and low O2 on intracellular [Ca2+] (167, 668). As outlined by Peers (592), a thorough quantitative analysis of the relation between neurotransmitter release and intracellular [Ca2+] is required to shed more light on the mechanism of stimulus interaction in type I cells. Other neurotransmitters than dopamine alone should be implicated in such analysis because the increase in afferent carotid sinus nerve activity by hypercapnia and hypoxia is not tightly coupled to dopamine release (100, 351, 743). The O2 sensor may behave like a hemoglobin molecule and, analogously to the Bohr shift, O2 binding may be competitively inhibited by CO2/H+, explaining the finding that the maximal effect of a very high PCO2 (~270 Torr) was the same regardless of the PO2 (180 and references therein).

2. Separate perfusion of peripheral and central chemoreceptors

A) Dogs. In a majority of early studies utilizing selective perfusion of the carotid bodies, hypoadditive effects on ventilation of peripheral and central stimuli were reported (references in Ref. 5). Multiplicative O2-CO2 interaction at the level of minute integrated phrenic nerve activity was also reported (132). More recently, Smith et al. (707) showed a clear multiplicative action of peripheral O2 and CO2 on ventilation but also a much larger inhibiting effect on the HVR of systemic hypocapnia than of carotid body hypocapnia alone, thus not supporting a hypoadditive peripheral-central interaction. In the same animal model, it was recently shown that isolated carotid body perfusion with hyperoxic hypocapnic blood led to a substantial decrease in ventilation despite a considerable rise in PCO2, not only suggesting an important role of the carotid body in eucapnic/normocapnic ventilation but also a hyperadditive peripheral-central interaction (77).

B) Goats. Awake goats subjected to selective carotid body perfusion showed O2-CO2 multiplication at the level of both carotid bodies and ventilation without any peripheral-central interaction other than simply additive (163, 164, 165). In awake goats instrumented for ventriculocisternal perfusion, carotid body stimulation with NaCN increased ventilation more at a relatively alkaline perfusate pH, possibly suggesting negative peripheral-central interaction. However, in this study an alkaline cerebrospinal fluid pH was associated with much higher Pco2 levels (708), thus almost inevitably leading to more intense central stimulation and potentially to simple additive central effects consistent with the findings in goats subjected to selective carotid body perfusion (163, 164, 165).

C) Cats. Anesthetized cats subjected to artificial brain stem perfusion did not show any central-peripheral interaction other than simply additive (314, 793). As in awake goats and dogs (163, 707), selective manipulation of peripheral gas tensions showed a multiplicative O2-CO2 effect on ventilation (314, 793). These findings might not entirely be compatible with those in the anesthetized cat showing that, with a constant level of hypoxia and a linear CO2 response of the carotid sinus nerve, CO2 had a smaller effect on ventilation below the eucapnic Pco2 than above it, suggesting some form of hyperadditive peripheral-central interaction (422). The disparity between the findings in anesthetized and awake cats (awake animals...
showing a negative peripheral-central interaction of tidal volume; Refs. 575, 576) cannot be explained by other than unidentified influences of anesthesia and/or arousal effects of hypoxia during wakefulness.

D) RATS. Utilizing an in situ dual perfusion system in the rat to separately perfuse peripheral and central chemoreceptors, Day and Wilson (172) found a hypoadditive effect of the central PCO2 on the hypoxic phrenic amplitude response. Perhaps this may explain the large HVR in this species at hypocapnic but not hypercapnic PaCO2 levels (145).

3. Dynamic end-tidal forcing

In both animals and humans, peripheral-central interaction can be studied using end-tidal forcing (DEF), enabling one to estimate separate gains of the central (Gc) and peripheral (Gp) chemoreflex loops, respectively (175, 747; see sect. vi). In the cat, Gc and Gp (assessed with both DEF and artificial brain stem perfusion) are independent, indicating no central-peripheral interaction other than additive (175, 314, 793). In addition, in the cat, O2-CO2 interaction takes place at the level of the carotid sinus nerve (234, 423), so the increase in ventilatory CO2 sensitivity with hypoxia in this species seems entirely due to an increased O2-CO2 interaction, compatible with an increased Gp but unaltered Gc in overall hypoxia (e.g., Refs. 779, 793). In young piglets, hypoxia increased Gp but did not alter Gc (845). In humans, an unchanged Gc with hypoxia was not always observed, with some studies showing a small but significant rise (226, 227) but others reporting no significant change (56, 156, 591). When Gp was lowered by inhalation of subanesthetic concentrations of volatile anesthetics, Gc did not change, not supporting an other-than-additive central-peripheral interaction (161, 796, 797).

4. Dynamic end-tidal forcing in carotid body resected subjects

Bellville (56) reported an ~50% lower central chemosensitivity in carotid body-resected subjects compared with healthy controls, indicating that a peripheral-central interaction other than simply additive cannot be excluded. One to 26 six years after bilateral removal of the carotid bodies due to paraganglioma, subjects displayed a lower central CO2 sensitivity than an age- and sex-matched control group (227). Three days after carotid body resection (due to hereditary paraganglioma), three patients showed a substantially reduced central CO2 sensitivity that gradually recovered in the following 1–3 years (154). In the anesthetized cat, carotid sinus nerve transection did not reduce central CO2 sensitivity in an acute setting (314). Thus removal of a tonic input from the carotid bodies could lead to a resetting of central CO2 sensitivity that needs time to develop. Whether such a relatively slow process may also be responsible for the lower CO2 sensitivity and higher resting arterial PCO2 after carotid body denervation in goats and dogs remains to be established (579, 663).

5. Other approaches used in humans

In earlier studies, so-called withdrawal tests were performed, suddenly removing a central or peripheral stimulus keeping the background peripheral or central gas tensions, respectively, at constant levels (reviewed in Ref. 150). Underlying assumptions such as lack of activity and absence of undershoots in carotid sinus nerve discharge in (mild) hyperoxia make these studies difficult to interpret (e.g., Ref. 325). Other workers compared transient and steady stimuli assuming that responses to the former originate at the peripheral chemoreceptors and those to the latter at both peripheral and central sites (see Ref. 206 for earlier references). The role of HVD could not always be taken into account, as well as the possible existence of an interaction component with a fast time constant (e.g., Ref. 656).

One way to study peripheral-central interaction is to expose subjects to peripheral stimuli at different central PCO2 levels. Robbins (656) compared the effects of a step decrease in end-tidal CO2 performed just 30 s after imposing a step decrease in end-tidal CO2 with those performed at constant steady-state end-tidal CO2. In two of three subjects, the response was larger under the former condition, suggesting a more-than-additive central-peripheral interaction (alternative explanations were left open; Ref. 656). However, St Croix et al. (724) could not reproduce this. Clement et al. (136) found no evidence for different responses to isocapnic changes in arterial pH induced by infusion of acid and bicarbonate performed at quite different background PCO2 levels. With the assumption that with acid-base changes the peripheral chemoreceptors respond uniquely to changes in arterial [H+], this seems unlikely because it would imply an almost infinitely high sensitivity in a metabolic acidosis with a pH of ~7.3 ([H+] ~50 nM) which, for example, can be induced by the carbonic anhydrase inhibitor acetazolamide (775, 778). To investigate this further, bicarbonate was infused isocapnically, and
this yielded two isocapnic and two virtually isohydric situations with different hypoxic sensitivities as shown in Figure 7. From these data (775), we can draw several conclusions. First, O2 sensitivity increases with an isocapnic rise in arterial [H+], and this is presumably due to interaction at the carotid bodies (cf. points c and f and b and d in Fig. 7). Second, although with acid-base alterations extracellular H+ may be the adequate stimulus to the carotid bodies, there is no unique relationship between hypoxic sensitivity and arterial [H+]. Points a and f are virtually isohydric points, and the lower hypoxic sensitivity in point a is presumably due to the lower P CO2 in the carotid bodies. See text for further explanation. Data are means ± SE from 8 young healthy volunteers. [Adapted from Teppema et al. (775).]

Figure 7. Relationship between hypoxic ventilatory response and arterial H+ concentration in humans. Hypoxic sensitivity, defined as ∆Vp/∆log PaO2, as a function of arterial [H+]. The right line represents the condition with normal arterial bicarbonate concentration in the arterial blood. The acute hypoxic response (i.e., the response 3 min after a step decrease in end-tidal O2) is measured at three constant levels of arterial P CO2 (numbers are arterial P CO2 values). There is a sound linear relationship between hypoxic sensitivity and arterial H+ concentration indicating a powerful O2/CO2-H+ interaction at ventilation level. When subjects are given intravenous bicarbonate (left line), the line shifts to lower values of arterial [H+] in an approximately parallel way. Thus apparently hypoxic sensitivity is not a unique function of the arterial [H+]. Points a and f are virtually isohydric points, and the lower hypoxic sensitivity in point a is presumably due to the lower P CO2 in the carotid bodies. Acetazolamide, a moderately permeable carbonic anhydrase inhibitor, will not have penetrated into the brain in significant amounts 1 h after administration of a low dose. About 1 h after infusion of 4 mg/kg, the O2-CO2 interaction at the carotid bodies cannot be made. In addition, an influence of developing HVD in these subjects cannot be excluded even though the hypoxic tests did not last 5 min: at the end of the hypoxic test in moderate hypocapnia (in the absence of an HVR), subjects ventilated less than in the prehypoxic period suggesting HVD (144).

Yang et al. (863) introduced a method for estimating the dynamic response to simultaneous changes in P CO2 and P O2 in humans, and their data could be well described with a two compartment model, one comprising the slow CO2 and the other the fast O2-CO2 interaction component. The interaction could be entirely modeled by the fast component attributed to the peripheral chemoreceptors, thus not supporting a significant central O2-CO2 interaction and/or more than simply additive peripheral and central effects.

Agents not crossing the blood-brain barrier and not influencing central CO2 sensitivity could be used to investigate possible selective effects on the carotid bodies. Acetazolamide, a moderately permeable carbonic anhydrase inhibitor, will not have penetrated into the brain in significant amounts 1 h after administration of a low dose. About 1 h after infusion of 4 mg/kg, the O2-CO2 interaction in healthy subjects was completely abolished, and this was reversible with antioxidants (Fig. 8; Ref. 777). Infusion of 500 mg in humans did not change hyperoxic CO2 sensitivity, indicating an unaltered central ventilatory CO2 sensitivity (750). With the assumption of no central effects of acetazolamide, these findings suggest that in humans, the O2-CO2 interaction entirely resides in the carotid bodies. A usual clinical oral dose of acetazolamide does not affect the interaction, indicating that its effect depends on the dose and route of administration (750, 775). Animal data suggest that analogous to its effect on hypoxic pulmonary vasoconstriction (749), the inhibitory action of acetazolamide on the HVR may occur independently from carbonic anhydrase inhibition (776).
6. Conclusion

In all species investigated, \( \text{O}_2 \) and \( \text{CO}_2 \) have multiplicative actions on carotid body output both when awake and anesthetized, but the interaction site within type I cells has not yet been established. The existence of a multiplicative \( \text{O}_2-\text{CO}_2 \) interaction on ventilation depends on the species, state of consciousness, and the peripheral-central interaction mode. Generally, humans show \( \text{O}_2-\text{CO}_2 \) multiplication, but it appears difficult to characterize the mode of peripheral-central interaction, although there is no conclusive evidence for hypoadditive central and peripheral effects. Direct anatomical connections between the first relay station of the peripheral chemoreceptors in the NTS and central chemoreceptors in the retrotrapezoid nucleus (RTN) as described for the rat (753), may render a clear separation of central and peripheral contributions elusive. In addition, a subset of \( \text{CO}_2 \) chemoreceptors in the RTN receives an inhibitory input from (mainly slowly adapting) pulmonary stretch receptors (525), providing another complicating factor in the interpretation of data from intact as well as vagotomized animals.

E. Carotid Body Resection and the HVR

Resection of the human carotid bodies was performed for a variety of reasons. Both unilateral and bilateral resections were carried out in patients with severe bronchial asthma and chronic obstructive pulmonary disease. Although the main goal for resection in these patients was often achieved (that is, the alleviation of breathlessness), the therapy is controversial and currently not practiced on a wide scale (326, 475, 812). Another reason for carotid body resection is the development of local tumors and/or hyperplasia that, for example, may arise from genetic defects (see sect. iv) and from chronic hypoxemia related to cyanotic heart disease, pulmonary disease, and high altitude (154, 312). Inadvertent loss of carotid body function may arise from surgical procedures in the neck region, such as carotid endarterectomy and neck dissection as part of the treatment of head and neck cancer (524, 812).

Both animal and human studies show immediate hypoventilation, respiratory acidosis, and loss of the hypoxic drive in response to bilateral carotid body resection (Fig. 5; Refs. 154, 473, 474, 492, 570, 664). In a small human population tested before and after bilateral removal of the carotid bodies for tumors due to a mutation in the SDHD gene, end-tidal \( \text{P}_{\text{CO}_2} \) increased by 8 Torr within the first day after resection (154). Similar observations were made in awake rats over a 75-day period after carotid body excision (570). These data indicate that the carotid bodies are essential in maintaining normal ventilation during air breathing. Especially in the neonatal period, elimination of the carotid bodies can have profound effects on the control of breathing, depending on the denervation technique used and the developmental period in which it is performed (238 and references cited therein). Partial restoration of the hypoxic drive is observed in some species (rat, goat, cat, pony, piglet) and is most likely in neonatal animals (69, 473, 474, 492, 710). The rate of recovery is species dependent and ranges from weeks (piglet, rat) to months (goat) to years (pony). The compensatory mechanisms of the partial recovery of the hypoxic drive in these animal studies remain unknown. Potential mechanisms include reinforcement of aortic bodies, activation of medullary oxygen-sensing neurons, chemosensory input from secondary glomus tissue in the head/neck region (such as the vagal body in the

![Diagram](https://via.placeholder.com/150.png?text=Diagram)
neck), regeneration of sensory terminals of the sinus nerve, and/or plastic changes in the central respiratory neuronal network. In humans, we were unable to observe any recovery in the HVR over a period of 4 years after bilateral removal of the carotid bodies (154). Similar observations were reported by Wade et al. (812) in patients up to 8 years postsurgery. Honda et al. (326) did show some restoration of the HVR in patients two decades after bilateral carotid body resection, although this effect was dependent on the specific hypoxic test employed.

F. Chronic Hypoxia and the HVR

1. Ventilatory acclimatization to chronic hypoxia

We define chronic hypoxia as exposure to hypoxia or high altitude lasting several hours to days, weeks, or months. A hallmark of adaptation to high altitude is a gradual rise in ventilation with a time course depending on the altitude (ventilatory adaptation to high altitude, VAH). In humans, at very high altitudes (8,000 m and higher), this may take at least 30 days (839). At less extreme altitudes, complete VAH requires ~10 days (see Ref. 68 and references cited therein), although at 3,800 m ventilation appeared not to have reached a plateau yet after 12 days (683). Dogs, goats, and ponies acclimatize much faster and reach complete adaptation after 3, 4-6, and 30 h, respectively (references in Ref. 68). When exposed to a simulated altitude of ~4,600 m, cats show a large decrease in arterial PCO2 after 48 h (809), but acclimatization may not yet be complete after this period in this species since at 5,500 m arterial PCO2 continued to fall for 10-12 days (774). Qualitatively, in the rat, VAH is rather similar to that in humans albeit with a somewhat faster time course, and this species is considered an adequate animal model for the adaptation in humans (569).

Initially it was suggested that VAH could be attributed to changes in arterial and later to brain stem acid-base status, but eventually it became clear that alterations in acid-base status during VAH should be considered the consequence of ventilatory changes rather than the cause (178). Here we do not focus on studies aimed at elucidating the role of the central chemoreceptors in VAH, and the reader is referred to excellent reviews on this subject (68, 540, 543). Briefly, there is no conclusive evidence for an essential role of the central chemoreceptors, for example, because the time courses of medullary extracellular fluid pH and VAH do not run in parallel (534, 543). Future studies are warranted, however, to unmask a possible role of central chemoreceptors in VAH for a reason not anticipated before: during hypoxia in the rat, central chemoreceptors in the retrotrapezoid nucleus are strongly activated via a glutamatergic pathway from carotid body projection areas in the NTS (753). Thus, during chronic hypoxia, these neurons may receive a time-dependent progressively increasing synaptic input that may overcome inhibitory influences of a lower PCO2 and may thus contribute to VAH.

2. VAH is associated with an increase of the HVR

Studies in the last two decades have convincingly shown that VAH is associated with a time-dependent augmentation of the HVR that at least partly is due to an increased carotid body sensitivity. Initial studies in dogs, ponies, goats, and rats had already demonstrated that carotid body denervation abolished or at least minimized VAH (reviewed in Ref. 68). Below we summarize the most important studies in goats, cats, rats, and humans showing the crucial role of both the carotid bodies and the CNS in mediating an increase in the HVR in chronic hypoxia.

1. Animal studies

In goats (mainly awake) with vascularly isolated carotid bodies, Bisgard, Forster, and co-workers collected many data on the mechanism of VAH that can be summarized as follows (66, 67, 105, 200, 216, 546, 836; further references in Ref. 68). 1) Animals exposed to chronic hypoxia show complete VAH within 4-6 h. 2) Isolated CNS hypoxia and/or hypocapnia do not evoke VAH, indicating that it must have a peripheral origin. 3) VAH requires intact carotid bodies that are perfused by hypoxic blood; isolated carotid body hypcapnia results in a rapid sustained rise in ventilation but does not induce the time-dependent fall in arterial PCO2 typical for VAH. 4) Both chronic poikilocapnic and isocapnic carotid body hypoxias increase the isocapnic acute hypoxic response (AHR), indicating that the enhanced sensitivity develops regardless of the PCO2 at the carotid bodies. 5) During isocapnic carotid body hypoxia for 6 h, carotid sinus nerve afferents of anesthetized goats show a progressive, time-dependent increase in impulse frequency that quantitatively can account for VAH.

When exposed to a barometric pressure of 460-440 Torr for 2-3 days, cats demonstrated a large time-dependent drop in end-tidal CO2, increases in both the HVR, and afferent carotid sinus nerve activity as well as an augmented isocapnic carotid body sensitivity to hypoxia (771, 808). Animals exposed to 10% O2 exhibited a higher isocapnic carotid sinus nerve sensitivity to hypoxia after 21 days but not during the initial few hours (34). In animals kept at a constant end-tidal O2 of 30 Torr for 7 h, nerve activity started to increase after 3 h (181). However, Lahiri et al. (428) were unable to demonstrate an increase in afferent nerve sensitivity to hypoxia after acclimatization to 10% O2 for 21-24 days. Finally, Tatsumi et al. (768), after keeping cats at 375 Torr for 3-4 wk, reported a time-dependent decrease in end-tidal CO2 along with a decreased HVR and isocapnic hypoxic carotid sinus nerve
sensitivity. An altitude-dependent decrease in HVR along with VAH had already been reported in earlier studies (e.g., Ref. 774; further references in Ref. 68). Thus, after a few days of hypoxia, cats appear to have an augmented HVR and an increased carotid body sensitivity, but after more than 2–3 wk, they show both a blunted HVR and carotid body sensitivity. So, perhaps an increase in carotid body sensitivity is sufficient for VAH in the first few days, but other mechanisms manifest after 2–3 wk.

The rat develops an augmented poikilocapnic HVR and ventilation in the initial few days of chronic hypoxia followed by a gradual return of the HVR to control or even slightly below at the end of the first week (51, 91, 252, 811). However, a fast “blunting” of the HVR in the rat has not been demonstrated very convincingly, for example, because in all these studies, it was measured under poikilocapnic conditions. Rather, it may be a manifestation of a gradual attenuation of acclimatization as also occurs in other species, but without blunting of the HVR (68). Aaron and Powell (1) showed a clear increase in isocapnic HVR in rats after 7 wk at 380 Torr. Note that after acclimatization this enhanced HVR was measured at a constant arterial P CO2 that was lower than in control by as much as 6–8 Torr, whilst both control and acclimatized animals showed a positive O2-CO2 interaction (1). These authors also compared isocapnic HVRs of acclimatized animals with that of controls that were made slightly hypercapnic to achieve matching of minute ventilation (613). In control animals, the constant arterial P CO2 was kept at a level that was higher than in acclimatized animals by ~9 Torr, but despite this, the HVR in the latter was much higher, suggesting that either the carotid bodies had undergone an impressive increase in O2 sensitivity and/or that the translation of afferent carotid body activity into ventilatory output (CNS gain) was greatly facilitated. It would be interesting to compare HVRs at equal hypercapnic P CO2 levels in control and acclimatized rats, because it cannot be excluded that a negative O2-CO2 interaction (145, 172) may have played a role in the observations of Powell et al. (613).

Dwinell and Powell (201) found a large increase in the CNS gain of a standard electrical input from the carotid sinus nerve in acclimatized rats (380 Torr, 1 wk). However, cats exposed to 440 Torr for 48 h developed both a greater isocapnic HVR and hypoxic carotid sinus nerve response but did not show an increased CNS gain (808). In goats, the effect of NaCN (assumed to cause maximal carotid body stimulation) on ventilation was not different before and after 4 h of isocapnic or poikilocapnic hypoxia (215), but this does not preclude changes in CNS gain during prolonged hypoxia in this species (615). Thus, in the rat, the most frequently used animal model to study VAH, an increased central gain has clearly been demonstrated, while it cannot be excluded to exist also in humans, goats, and ponies (references in Refs. 178, 615).

2. Studies in humans

In Table 3, we have collected studies mainly performed over the last 15 years (for a review of earlier studies, see Ref. 68). Despite all the distinct exposure paradigms (stimulus level and duration, hypobaric hypoxia, or just lowered inspired O2), techniques and analysis methods that make quantitative comparisons very difficult if not impossible, the outcomes of all these studies have two things in common: an increase in resting and, if measured, hyperoxic ventilation and an augmented (acute) hypoxic ventilatory response that develops during the first 4–8 h of acclimatization.

Robbins (659) made considerable efforts to spell out the mechanisms of ventilatory adaptation to chronic hypoxia. Apart from a consistent increase in hyperoxic ventilation and an augmented acute response to hypoxia, he found the following: 1) in agreement with results obtained from goats, isocapnic and poikilocapnic hypoxias have the same effect. 2) Neither eucapnic nor hypocapnic passive hyperventilation evokes VAH or an enhanced HVR, indicating that hypocapnia or hyperpnea per se will not play a significant role. 3) The effect of hypoxia to induce the adaptations is due to the low P O2 rather than to low oxygen content. 4) The mechanism underlying the increase in the acute response to hypoxia is fast (observable within a few hours as in goats), sensitive (end-tidal O2 10 Torr below resting is sufficient to evoke it), independent of peripheral adrenergic and dopaminergic systems, and insensitive to general autonomic blockade (references in Table 3).

Chronic isocapnic and poikilocapnic hypoxia also alter peripheral ventilatory CO2 sensitivity. Using dynamic end-tidal forcing (see sect. vi) and sophisticated analysis methods, Fatemian and Robbins showed an increase in CO2 sensitivity that most likely can be attributed to an increase in sensitivity of the carotid bodies (e.g., Refs. 228, 229; further references in Table 3). In a study at 4,300 m (629), the amount of VAH correlated well with the magnitude of the HVR at sea level, also supporting an important role of the peripheral chemoreflex loop.

In a more recent study, Herigstad et al. (320) exposed subjects to 8 h of isocapnic hypoxia prior to an incremental exercise protocol. The exposure resulted in a decrease in end-tidal P CO2 that was essentially maintained at all work loads until exhaustion. According to the alveolar ventilation equation, this means that ventilation must have increased in proportion to metabolism. The data from this study also implied that an altered acid-base balance (isocapnia was maintained) or long-lasting increases in ventilation (days) are not required for this phenomenon to occur. Thus VAH augments the ventilatory response to exercise and in this sense resembles the effect of hypoxia or carotid body stimulants (references in Ref. 320). Exercise also augments the stimulatory effect of hypoxia.
### Table 3. Carotid body adaptations to chronic hypoxia

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects and Comments</th>
<th>Does the Adaptation Increase or Decrease CB Sensitivity or the HVR?</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurochemical adaptations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopaminergic system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Upregulation of TH</td>
<td>65, 614 and references cited therein</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>TH upregulation</td>
<td>Increase</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>CH augments HVR after 2 days at 4,600 m but domperidone fails to further increase it, suggesting dopaminergic downregulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>Prolonged hypoxia decreases HVR; domperidone restores it to control level, suggesting a role of DA upregulation in long-term adaptation</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>Dopaminergic system in efferent inhibitory fibers in the CSN is upregulated not supporting disinhibition of this system in CH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>After 5 and 2–3 wk of CH, domperidone restored “blunted” poikilocapnic HVR, attributed to increased CB DA content</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>1–2 days of CH attenuate dopaminergic inhibition of the HVR, but after 8 days it has increased compared to control</td>
<td>Increase and decrease, respectively</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>CB D2R decreased by 59% after 2 days of CH, but increased by 274% after 11 days</td>
<td>Increase and decrease, respectively</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>After 2 days of CH, CB DA decreased, but 2 days after termination of an 8-day lasting exposure it was higher than control</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>Male DA−/− mice failed to show VAH and augmented HVR after 2–8 days of CH</td>
<td>Increase, but note possible central effects</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Type I cells of juvenile rats show exaggerated basal DA release</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>The peripheral DA antagonist domperidone had equal effects in acclimatized and control animals, and local CB application of DA did not influence the time course and magnitude of VAH</td>
<td>Neither</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Effects of DA and domperidone did not change after 8 h of CH</td>
<td>Neither</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>CH for 24–36 h reduced the inhibitory effect of α1-adrenergic antagonism, suggesting downregulation of CB noradrenergic mechanisms</td>
<td>Increase</td>
<td></td>
</tr>
<tr>
<td>Various species</td>
<td>Upregulation of CB NE</td>
<td>Decrease</td>
<td>References in 65, 278, 818</td>
</tr>
<tr>
<td>Goat</td>
<td>CB sympathectomy did not alter the time course of VAH, the acute HVR and the inhibitory effects of NE and DA</td>
<td>Neither</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>VAH and CH-induced augmentation of the HVR are not affected by β-adrenergic and autonomc ganglion blockade</td>
<td>Neither</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>In CB preps from animals exposed to CH for 9–22 days, ACh had a larger excitatory effect on CSN discharge compared with control; however, the nAChR agonist mecamylamine failed to cause any reduction in hypoxia-induced increase in CSN activity</td>
<td>Increase</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>In type I cells cultured in normoxia mecamylamine did not alter basal DA release, but in those cultured in hypoxia for ~12 days it completely inhibited it, possibly explaining its failure to inhibit the hypoxia-induced rise in the preparation of He et al. (309)</td>
<td>Increase</td>
<td>355</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>Rat</td>
<td>In superfused CB preps from rats exposed to CH for 9–16 days, P2X2 purinergic but not nicotinic antagonists retained the ability to block hypoxia-induced rise in CSN activity, suggesting an altered purinergic/cholinergic balance; adrenergic discharge in CSN from acclimatized animals was blocked by purinergic blockers suggesting prolonged ATP release as its cause and hypothetically providing a mechanism for continuous hyperventilation upon return to normoxia from high altitude (deacclimatization)</td>
<td>Increase</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Rat</td>
<td>Upregulated in hypoxia</td>
<td>Decrease</td>
</tr>
<tr>
<td>Rat</td>
<td>In CB-CSN preps from rats exposed to CH for ≥16 days, L-NAME caused a progressively greater stimulation and an NO donor a larger inhibition</td>
<td>Decrease</td>
<td>308</td>
</tr>
<tr>
<td><strong>Substance P</strong></td>
<td>Cat</td>
<td>CB Substance P downregulated in animals exposed to 10% O₂ for 2 wk</td>
<td>Decrease</td>
</tr>
<tr>
<td><strong>Endothelin-1</strong></td>
<td>Rat</td>
<td>Upregulation of ET₁, and ET₂ receptors in type I cells from animals exposed to CH for ≥16 days. Selective inhibition of ET₁ receptors reduced basal CSN activity on day 0 by 11%, but halved it at day 16</td>
<td>Increase</td>
</tr>
<tr>
<td>Rat</td>
<td>During CH for 16 days, daily administration of the ET₂ receptor antagonist bosentan diminished the increase in hypoxic CSN activity and also greatly reduced CB enlargement and mitotic type I cell activity</td>
<td>Increase</td>
<td>129</td>
</tr>
<tr>
<td>Human</td>
<td>CH for 4 h increased plasma [ET-1], continuous infusion of ET-1 during a 4 h bout of CH exaggerated the increase in HVR</td>
<td>Increase, assuming a stimulatory effect of ET-1 on the CB</td>
<td>755</td>
</tr>
<tr>
<td><strong>Angiotensin II</strong></td>
<td>Rat</td>
<td>10% O₂ for 4 wk progressively increased CB angiotensinogen mRNA, upregulated AT₁ receptors and ACE, and increased CB sensitivity to ANG II</td>
<td>Increase</td>
</tr>
<tr>
<td><strong>Proinflammatory cytokines</strong></td>
<td>Rat</td>
<td>Upregulation of IL-1, IL-6, and TNF-α and their receptors in type I cells from rat exposed to 10% O₂ for 3, 7 and 28 days; after CH, the hypoxia-induced rise in [Ca²⁺] was enhanced by the cytokines</td>
<td>Increase</td>
</tr>
<tr>
<td>Rat</td>
<td>Rats exposed to a barometric pressure of 380 Torr for 4 wk showed increased CB [IL-1β] and [TNF-α] after 1 day, well before macrophage invasion; cytokine levels normalized after 28 days, except IL-6 due to increased production by the CB; ibuprofen and dexamethasone inhibited CH-induced increase in hypoxic CSN activity</td>
<td>Increase</td>
<td>455</td>
</tr>
</tbody>
</table>
### TABLE 3.—Continued

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects and Comments</th>
<th>Does the Adaptation Increase or Decrease CB Sensitivity or the HVR?</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin</td>
<td>Rat Exposing rats to 10% O₂ for 4 wk increased CB melatonin receptor mRNA</td>
<td></td>
<td>785</td>
</tr>
</tbody>
</table>

**Adaptations of ion channels and membrane currents**

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects and Comments</th>
<th>Does the Adaptation Increase or Decrease CB Sensitivity or the HVR?</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxi-K channels</td>
<td>Rat Decrease in K⁺ current density but not that caused by maxi-K channels, proposed to result in a relative larger contribution of the latter to the resting membrane potential and an increased hypoxia-induced catecholamine release</td>
<td>Increase</td>
<td>114</td>
</tr>
<tr>
<td>Sodium current</td>
<td>Rat Higher current density in type I cell isolated from 5- to 12-day-old rat and cultured in hypoxia for 1–2 wk</td>
<td>Increase</td>
<td>726, 727</td>
</tr>
<tr>
<td>“Oxygen-sensitive and -insensitive” Kᵥ channels</td>
<td>Rabbit In type I cells cultured in hypoxia for 72 h, potassium channels responsible for an O₂-insensitive component of the K⁺ current were downregulated and those contributing to the O₂ sensitive component upregulated; thus K⁺ current inhibition by hypoxia was increased</td>
<td>Increase</td>
<td>368</td>
</tr>
<tr>
<td>Sodium channel Naᵥ1.1</td>
<td>Rat CB from animals exposed to 10-11% O₂ for 1 wk contained type I cells with upregulated voltage-gated sodium (Nav1.1) channels; the sodium channel activator veratridine caused a severalfold greater increase in CA release in these cells, compared with those from control animals</td>
<td>Increase</td>
<td>106</td>
</tr>
<tr>
<td>Gap junctions</td>
<td>Rat Exposure to an ambient pressure of 380 Torr was followed by increased expression of the gap junctional protein connexin43 in type I cells and petrosal ganglion neurons</td>
<td>Increase?</td>
<td>127</td>
</tr>
</tbody>
</table>

**Morphologic adaptions of the carotid body**

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects and Comments</th>
<th>Does the Adaptation Increase or Decrease CB Sensitivity or the HVR?</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various species</td>
<td>Organisms living at high altitude have enlarged CB</td>
<td>Increased in short-term CH but decreased in prolonged hypoxia</td>
<td>19, 209, 419, 818</td>
</tr>
<tr>
<td>Rat</td>
<td>CH induces vascular remodeling, endothelial proliferation, and vasodilation</td>
<td>Unknown</td>
<td>135, 419, 420, 784, 818</td>
</tr>
<tr>
<td>Rat</td>
<td>Hyperplasia of type I cells during the first week of CH, no further increase thereafter; newly formed cells can survive for 4 mo</td>
<td>Increase</td>
<td>818, 819</td>
</tr>
<tr>
<td>Mice</td>
<td>Exposing mice to CH for 20 days resulted in cell division in CB after 1 day, but new type I cells appeared after 5 days. Initially type II cells start to divide and are replaced by “progenitor,” i.e., stem cells that differentiate into type I cells with normal electrophysiological features. Upon return to normoxia, 50% of the type I cells are freshly formed and the total amount gradually returns to normal</td>
<td>Increase</td>
<td>581</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; ACE, angiotensin converting enzyme; ANG II, angiotensin II; AT₁ receptor, angiotensin I receptor; CH, chronic hypoxia; CA, catecholamine; CSN, carotid sinus nerve; CB, carotid body; DA, dopamine; D₂R, dopamine 2 receptor; ET, endothelin; L-NAME, N\(^\text{G}\)-nitro-L-arginine methyl ester, an inhibitor of NO synthase; HVR, hypoxic ventilatory response; IL, interleukin; NE, norepinephrine; nAchR, nicotinic acetylcholine receptor; NO, nitric oxide; TH, tyrosine hydroxylase; TNF, tumor necrosis factor; VAH ventilatory adaptation to chronic hypoxia or high altitude.

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of hypoxia on ventilation, but this interaction is reduced by a prior exposure to chronic hypoxia, and this phenomenon will be one of the determinants of the magnitude of the HVR at high altitude (848).

A decrease in intercept of the hyperoxic CO₂ response curve was not observed by Fatemian and Robbins (228, 229), suggesting that the increase in resting and hyperoxic ventilation is due to a higher (peripheral) CO₂ sensitivity. In one study, a leftward shift of the ventilatory CO₂ response curve was found after as long as 75 h of poikilocapnic but not isocapnic hypoxia (148). Studying the effect of chronic hypoxia with modified rebreathing (see sect. VI) yields a decrease in intercept of both the hypoxic and hyperoxic CO₂ response curves at high altitude, but, possibly related to variation in altitude and acclimatization period, no consistent increase in peripheral CO₂ sensitivity (9, 719).

Two major mechanisms, to be discussed in the following paragraphs, may contribute to the augmented HVR in chronic hypoxia: an augmented carotid body sensitivity and, as particularly studied in the rat, an increase in CNS gain. Animal studies do not provide direct evidence for an important role of the sympathetic nervous system (e.g., Ref. 672). In humans, prolonged hypoxia causes a large increase in sympathetic activity, but how this may influence the HVR is unknown (107, 300). Also, note that the chronic hypoxia-induced HVR augmentation in humans was reported to be unaltered after autonomic ganglion blockade (452). Neonates and adults adapt differently to chronic hypoxia. This is schematically shown in Figure 9.

3. Chronic hypoxia increases carotid body sensitivity

Chronic hypoxia elicits morphological, neurochemical, and membrane-electrophysiological adaptations in the carotid bodies that act together to increase hypoxic sensitivity. These adaptations occur in parallel, so their separate contribution to the increase in carotid body gain is difficult to assess. This is especially true for the contribution of neurotransmitters and/or modulators, some of which have a controversial role in the carotid bodies. For example, the dopaminergic system seems to be upregulated in chronic hypoxia, but although dopamine is generally considered an inhibitory neurotransmitter (mainly based on observations on the effects of exogenous applied agonists and antagonists), the significance of this adaptation is uncertain because endogenous dopamine may well have excitatory effects on the carotid bodies (for a discussion on the controversial role of dopamine in the carotid body, see Refs. 278, 283, 869). Both an inhibitory and excitatory role has also been suggested for norepinephrine (references in Refs. 68, 278). In Table 4, we have summarized recent studies on various adaptations of the carotid bodies to chronic hypoxia. We have added a column containing the suggested effects of single adaptations on carotid body sensitivity, but we emphasize that with regard to neurotransmitters these must be considered hypothetical, because what eventually matters is the total balance between excitatory and inhibitory transmitters. In a recent review, Prabhakar (621) has proposed the simultaneous presence of excitatory and inhibitory transmitters to result in a “push-pull” mechanism between both that, rather than causing exhaustion in the absence of inhibitors, produces sustained activation in their presence. This would be especially effective in the adaptation chronic hypoxia (621).

4. Increase of CNS gain during chronic hypoxia

An increased CNS gain as observed in the rat could result from neuroplasticity of the brain stem respiratory circuitry that processes the afferent input from the carotid bodies. Below we discuss some mechanisms potentially underlying the increase in CNS gain in chronic hypoxia.

A) CATECHOLAMINERGIC SYSTEM. Rats challenged with 10% O₂ for 1 wk showed a reduced brain dopamine turnover and an increased carotid body dopamine content (571). In rats exposed to chronic hypoxia for 2 wk, Schmitt et al. (685) found a positive correlation between the rise in ventilation and tyrosine hydroxylase (TH) protein level in the caudal NTS, i.e., the preferential projection area of chemosensitive fibers. The same area, containing the A2 noradrenergic cell group, displayed an upregulation of TH after 1 wk of chronic hypoxia, but this effect was reversed after 2 and 3 wk of exposure, respectively (722). When administered in the NTS, dopamine has a stimulatory influence on ventilation (for references, see Ref. 345), and thus an increased tyrosine hydroxylase production may facilitate respiratory output in chronic hypoxia. In male normoxic mice systemic droperidol, a dopamine antago-

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**FIG. 9.** Comparison of chronic hypoxia in neonates and adults. Effects of chronic hypoxia in neonates, within the critical time window for developmental respiratory plasticity, compared with those in adults. Question marks concern unresolved or controversial issues. Vᵢ, inspired ventilation. For explanations see text.
### TABLE 4. Chronic hypoxia in humans

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<td>Progressive isocapnic rebreathing at PET_{CO2} of ambient air breathing</td>
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TABLE 4.—Continued

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<td>iH: (P_{ETCO_2}) 50 Torr, 8 h, followed by iv infusion of the ganglion blocker trimethaphan</td>
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\(CaO_2\), arterial oxygen content; \(iH\), isocapnic hypoxia; \(pH\), poikilocapnic hypoxia; \(iAHR\), isocapnic acute hypoxic response; \(CH\), chronic hypoxia; \(iHVR\), isocapnic hypoxic ventilatory response; \(DEF\), dynamic end-tidal forcing; \(NHC\), normobaric hypoxic chamber; \(V_0\), inspired minute ventilation; \(P_0\), barometric pressure; \(P_{ETCO_2}\), end-tidal \(P_{CO_2}\); \(P_{ETO_2}\), end-tidal \(P_{O_2}\); \(SpO_2\), arterial hemoglobin oxygen saturation.

nism that penetrates into the brain, increased ventilation, suggesting a predominance of the inhibitory carotid body dopaminergic system on respiratory output in normoxia (572). However, mice kept in 10% \(O_2\) for 10 days responded to droperidol with a decrease in ventilation indicating a dominance of the central excitatory dopaminergic system in hypoxia (572). This seems consistent with data from mice lacking the dopamine \(D_2\) receptor \((D_2^-R^-)\) that failed to show VAH and an augmented HVR after 8 days of acclimatization, an effect that was not yet demonstrable after 2 days (343). These studies suggest that a shift from peripheral to central dopaminergic dominance may contribute to VAH. How this can be reconciled with an increase in carotid body dopamine content in prolonged hypoxia remains to be seen. Upregulation during chronic hypoxia of TH and norepinephrine was also observed in the in the locus coeruleus of rats (684), but its contribution to VAH is difficult to assess because this region is the source of a widespread noradrenergic network in the brain.

B) NMDA RECEPTORS. During hypoxia, glutamatergic NMDA and non-NMDA receptors in the caudal NTS play a crucial role in the transmission of afferent impulses from the carotid body (287, 516, 565, 801 and references therein). Reeves et al. (634) showed an early upregulation of the \(NR_1\) subunit of the NMDA receptor in the dorsal caudal brain stem of rats which had resolved after 30 days of chronic hypoxia. The caudal NTS of rats exposed to 10% \(O_2\) for 7–8 days contained higher levels of the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit glutamate receptor 2 (Glur2), which was thought to contribute to an enhanced glutamatergic synaptic transmission of arterial chemoreceptors inputs into the NTS (873). NMDA receptors in the medulla of mice were upregulated following exposure to 10% \(O_2\) during 2 wk (210; see also sect. \(V\)). In rats exposed to 10% \(O_2\) for 9 days, the NMDA antagonist MK-801 had a larger inhibitory effect on hypoxic ventilation than in unacclimatized controls (638).

C) PDGF-\(\beta\) RECEPTORS. Administration of PDGF-BB in the NTS decreased the early HVR in rats, and mutant mice heterozygous for the PDGF-\(\beta\) receptor showed a reduced HVD (see also sect. \(mD\)). This led to the suggestion that downregulation of PDGF-\(\beta\) receptors during chronic hypoxia might contribute to VAH (287, 289). During 14 days in 10% \(O_2\), rats demonstrated a posttranscriptional downregulation of PDGF-\(\beta\) receptor protein in the dorso-caudal brain stem after 7 days, significantly correlated with VAH, while the attenuation of the HVR by local PDGF-BB application in the NTS decreased over time and actually was absent from day 7 on (14). In addition to PDGF, other growth factors such as brain-derived neurotrophic factor (BDNF), located in petrosal ganglion neurons and possibly acting postsynaptically, could also contribute to plastic changes within the NTS during chronic hypoxia.
D) NO-RELATED MECHANISMS. As discussed in sections III C3, NO is an important central mediator of the HVR with a stimulatory effect on the HVR. Thus upregulation of neuronal NOS could also enhance CNS gain. Mice exposed to 10% O2 for 2 wk had higher medullary neuronal NOS levels, which seemed related to VAH but not to the increased HVR: the former was reduced while the latter remained unaffected by a specific neuronal NOS inhibitor (210). Upregulation of neuronal NOS (but not the endothelial isoform) was also shown in peripheral and central neurons in rats after 12–24 h in hypobaric hypoxia (618). The hypoxia-induced rise in intracellular NO in the NTS may be one of the secondary consequences of NMDA receptor activation (references in Ref. 287). Thus, in the NTS, a feed-forward mechanism could exist between NMDA receptor activation and release of NO to enhance the CNS gain (502). The above-cited work by El-Hasnaoui et al. (210) does not directly support this hypothesis. Rats exposed to 12% O2 for 2 wk and receiving a continuous intracerebroventricular infusion of the NOS inhibitor L-NAME showed a lower normoxic ventilation than controls after this period, indicating a reduced VAH. However, their HVR was indistinguishable from that in acclimatized controls not receiving the enzyme inhibitor (688). These scarce data raise the impression that upregulated NO in the medulla may contribute to VAH but not to the increase in HVR.

E) ERYTHROPOIETIN. Mice overexpressing human erythropoietin (Epo) in the brain without elevation of plasma Epo and hematocrit (Tg21 mice) were reported to show larger increases in ventilation and HVR in chronic hypoxia, even after carotid sinus nerve section (716). Female (but not male) mice with increased peripheral and central Epo expression (Tg6 mice) were reported to have an augmented HVR (717). However, another study in Tg6 mice (sex not mentioned) reported no difference in HVR compared with wild types; after acclimatization, VAH and an augmented HVR were clearly present in wild types but totally absent in the Tg6 genotype, and this was suggested to be related to upregulated carotid body dopaminergic mechanisms (804). Finally, infusion into the brain of the soluble Epo receptor, that normally is downregulated in chronic hypoxia thus increasing Epo bioavailability, reversed VAH in normal mice (715).

F) CENTRAL OXYGEN-SENSING NEURONS. In the initial phase of VAH (10% O2 for 4–5 days), O2-sensitive cells in the RVLM displayed an increased O2 sensitivity in vitro that in later stages (9–10 days) of acclimatization was offset, possibly due to an altered neurotransmitter balance or other processes decreasing membrane excitability (554). In both normoxia and hypoxia, the C1 region and the pre-Bötzheimer complex show a constitutive expression of HO-2 (501). Placing rats in 10% O2 for 10 days induced HO-1 but not HO-2 in the ventral medulla. At this stage, however, the significance of this observation is unclear since the role of the former isoform in O2 sensing by central neurons is unknown (501), whereas in unacclimatized condition, the isoform HO-2 seems to be required for oxygen sensing in neurons of the pre-Bötzheimer and C1 regions (153). These scarce data could suggest a contribution of central oxygen sensors to the increased CNS gain in chronic hypoxia. Isolated central hypoxia, however, does not lead to VAH in the goat (836).

G) EXPRESSION PROFILE OF ION CHANNELS IN THE NTS. Neurons in slice preparations from rat containing NTS neurons receiving monosynaptic input from the carotid bodies respond to hypoxia with an outward potassium current via activation of KATP channels (i.e., ATP-sensitive potassium channels that close in the presence of high ATP concentrations; Ref. 872). In slices from rats exposed to 10% O2 for 1 wk, this current was reduced, and this was possibly caused by an alteration of the expression profile of regulatory subunits of these channels. This then may have increased both the excitability of these neurons and CNS gain (872).

H) PLASTICITY-INDEPENDENT MECHANISMS TO INCREASE THE CNS GAIN. The brain contains high concentrations of O2-sensitive (human tandem P domain) TREK-1 potassium channels, predominantly in GABAergic inhibitory interneurons, that play a key role in setting the resting membrane potential (references in Ref. 511). In human embryonic kidney (HEK-293) cells, these background K+ channels are inhibited by low PO2, in a range relevant for brain tissue and also, in an additive way, by intracellular alkalosis (511). Inhibition of these channels in GABAergic inhibitory interneurons by mild alkalosis might then be of physiological significance especially at the background of hypoxia-induced alkalosis (511). In the rat, an additional way of increasing CNS gain could exist in a negative peripheral-central interaction (145, 172, 613; see sect. V D). The time-dependent fall in PO2 in the early acclimatization phase could augment the central response to carotid body hypoxia, thus increasing ventilation and the HVR. Isocapnia should then be expected to decrease the magnitude of these adaptations, but this has not been systematically investigated in the rat.

Many of the above modulations and adaptations in both the carotid bodies and CNS are undoubtedly the consequence of altered gene expression induced by HIF-1. Rats exposed to chronic hypoxia (0.5 atm, 3 wk) displayed accumulation of HIF-1α in neurons, astrocytes, ependymal cells, as well as endothelial cells and also showed upregulation of genes with hypoxia-responsive elements (126). HIF-1α+/− mice lack an increase in carotid sinuses nerve activity in response to hypoxia (not to NaCN) but increase their ventilation, which is initiated by the aortic bodies. Ventilatory adaptation to a 3-day bout of hypoxia does not occur in these animals (388). Chronic hypoxia induces carotid body expression of both HIF-1α, HIF-2α, and HIF-3α, which all contribute to morphologi-
nal and neurochemical adaptations (434, 584, 614, 784 for further references). Humans with an impaired degradation rate of HIF-1α in normoxia (Chuvash polycythemia; see sect. iv) have an exaggerated acute HVR and low resting PCO₂, much similar to VAH (712). The contribution of HIF-1 in both the carotid bodies and the CNS to VAH is recently reviewed by Powell and Fu (614).

Other transcription factors also are involved. For example, a role of activator protein-1 (AP-1) is indicated by the finding that mice lacking the fosB gene (a member of the fos family of immediate early genes), although having a normal HVR when kept in normoxia, do not exhibit VAH and an increase in HVR when exposed to chronic hypoxia (486).

Chronic hypoxia may also recruit additional oxygen sensing mechanisms that are silent in a normoxic condition.

G. Chronic Intermittent Hypoxia and the HVR

1. Episodic hypoxia induces phrenic and ventilatory LTF

In awake rats, dogs, and goats, the termination of episodic hypoxia, for example, a challenge with repeated hypoxia of short duration (mostly 1–5 min) interrupted by short periods of reoxygenation, is followed by a prolonged increase in ventilatory motor output, a phenomenon that is known as LTF (112, 506, 568, 792). Sustained hypoxia of short duration does not evoke the phenomenon (27). At the level of phrenic and hypoglossal motor outputs, LTF can be evoked by intermittent electrical stimulation of the carotid sinus nerve and/or continuous stimulation of medullary raphe obscurus neurons in anesthetized, (mostly) vagotomized animals (244, 305, 495, 512, 513) or by repetitive hypoxia (23, 245, 387, 504). LTF requires an intact serotonergic system with neurons in medullary raphe nuclei playing a crucial role (23, 506, 514).

We briefly summarize the main underlying mechanisms of phrenic LTF as far as they may also be relevant for (plastic changes of) the HVR in chronic intermittent hypoxia (references in Refs. 230, 447, 480, 481). During episodic hypoxia, raphe serotoninergic neurons in the medulla are activated resulting in serotonin release in the vicinity of brain stem respiratory neurons and spinal motoneurons. Each hypoxic episode triggers a release of 5-HT. One of the downstream cascades then activates several protein kinases that may phosphorylate glutamate receptor proteins, thus increasing synaptic strength by enhancing NMDA receptor currents. Some phosphates, on the other hand, may lay a constraint on LTF (references in Ref. 481). A key role in LTF is played by BDNF. During episodic hypoxia, the synthesis of this neurotrophin in the spinal cord increases; application of BDNF in the spinal cord of rats is sufficient to evoke LTF. In addition, when BDNF translation and new protein synthesis is prevented, episodic hypoxia does not induce LTF (28). BDNF is thought to act through the tyrosine kinase B receptor which then can lead to the facilitation of glutamatergic transmission via several pathways (28).

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Episodic hypoxia induces phrenic LTF, even without hypoxia. So the working model of episodic hypoxia-induced phrenic LTF is that it is mediated by a release of serotonin, the activation of NADPH oxidase, and increased production of BDNF and ROS with facilitation of glutamatergic synaptic transmission in the phrenic motor nucleus as the eventual result (27, 28, 230, 447, 480, 481 and references cited therein). In both artificially ventilated and spontaneously breathing rats, LTF is substantially reduced by NMDA antagonists injected in the phrenic motor nucleus (447).

2. Chronic intermittent hypoxia augments LTF, sensitizes the carotid bodies in animals, and increases the HVR

Following three 5-min episodes of isocapnic hypoxia, the phrenic neurogram of rats showed a somewhat increased baseline amplitude that further increased over time to become significantly greater than the prestimulus amplitude after 30 and 60 min, respectively, indicating LTF (448). Exposing rats to chronic continuous alternations of 5 min of hypoxia (11–12% O₂) and 5 min of air for 12 h/night for 7 days, i.e., chronic intermittent hypoxia, augmented the acute phrenic response to episodic mild hypoxia and, via a serotonergic pathway, increased LTF (448). This suggests that the chronic intermittent hypoxia had resulted in an augmented carotid body sensitivity to hypoxia and/or increased CNS gain. Electrical carotid sinus nerve stimulation now resulted in an enhanced activation of the phrenic nerve, indicating an increased CNS gain (448). In conscious rats, however, not all these findings could be confirmed at the level of minute ventilation because a similar chronic intermittent hypoxia protocol induced ventilatory LTF but no increase in the HVR (505, 506).

Similar to episodic hypoxia-induced LTF, the effect of chronic intermittent hypoxia to augment its magnitude is a NMDA receptor-related mechanism; however, the serotonin subtype involved may be a different one: a 5-HT₂ subtype in episodic hypoxia, and a 5-HT₄ and/or
5-HT_7 subtype in the chronic intermittent hypoxia-induced augmentation (447).

These chronic protocols with relatively long durations of mild hypoxia in rats were not directly intended to mimic the rapid and more severe hypoxic swings that are experienced by sleep apnea patients but were mainly aimed at studying the plasticity of the neuronal network shaping the HVR. Peng and Prabhakar (597) have extended the above observations on the effects of episodic hypoxia by imposing hypoxic episodes lasting seconds rather than minutes, a stimulus pattern more relevant to sleep apnea. They showed that conditioning rats with chronic intermittent hypoxia consisting of 15 s hypoxia alternated with 5 min of normoxia, 9 cycles/h for 8 h/day during 10 consecutive days, augmented the episodic hypoxia-induced phrenic LTF, which was absent in animals exposed to sustained hypoxia with a comparable cumulative duration (597). Combining chronic intermittent hypoxia conditioning with daily administration of a superoxide dismutase (SOD) mimic prevented this LTF augmentation (597). Subsequently, they showed in the rat that chronic intermittent hypoxia: 1) evoked episodic hypoxia-induced LTF in the carotid sinus nerve (“sensory LTF”) that was prevented with a SOD mimic; 2) reduced both aconitate activity in the carotid bodies and the activity of mitochondrial complex I; and 3) increased hypoxic but not hypercapnic sensitivity of the in vivo and in vitro carotid body, which was prevented by scavenging superoxide radicals and could be reversed by subsequent adaptation to normoxia (585, 595–597). This functional carotid body plasticity occurred without macroscopic morphological changes (585, 596).

Additional studies in mice with a partial deficiency for HIF-1α (HIF-1α^+/−) revealed that, in contrast to wild types, these animals did not respond to chronic intermittent hypoxia with increases in HVR and carotid body sensitivity. Also, the heterozygous animals did not accumulate ROS and showed no upregulation of HIF 1-α (600). Prabhakar and colleagues (536) proposed a working model of chronic intermittent hypoxia in which oxidative stress leads to ROS generation (presumably by increased NADPH oxidase activity and/or inhibition of mitochondrial complex I), upregulation of HIF-1 and induction of many genes by this transcription factor that together help to sensitize the carotid bodies. Other transcription factors and posttranscriptional phosphorylation of proteins also play an important role (414, 536).

NO could also be involved in chronic intermittent hypoxia-induced LTF and carotid body sensitization. Mice deficient in neuronal NOS have a blunted O_2 response (390); the effect of chronic intermittent hypoxia in these animals are unknown.

An increase in the sustained, but not acute, HVR was reported in rats after alternations of 10 and 21% O_2 every 90 s, continuously applied during 30 days (634). Challenging cats with alternations of 90 s hypoxia with ~5 min normoxia, 8 h/day for 2–4 days enhanced the in vivo basal discharge and hypoxic sensitivity of the carotid sinus nerve, upregulated carotid body ET-1 by a factor of ~10 and shifted the hypoxic response curve of their in vitro vascularily perfused carotid bodies to the right (643). Subsequently, a chronic intermittent hypoxia-induced upregulation of carotid body ET_B but not ET_A receptors was shown, and it was proposed that upregulation of the endothelin system would increase carotid body sensitivity by modulating blood flow (642). Recently, it was suggested that, similar to its facilitatory effect on synaptic transmission in the NTS, ET-1 may also stimulate the carotid body by augmenting the excitatory effect of glutamate (456). Rats exposed to chronic intermittent hypoxia for 3 wk showed upregulation of carotid body NR_1, and NR_2, NMDA receptor subunits, and in contrast to controls, carotid body baseline activity in these animals was enhanced by infusion of NMDA without altering the sensitivity to hypoxia. The NMDA antagonist MK-801 not only reversed this increase in baseline activity but also inhibited the stimulatory effect of ET-1 (456). So, although probably not directly involved in O_2 sensing, NMDA receptors may play a functional role in the adaptation of the carotid bodies to chronic intermittent hypoxia.

Chronic intermittent hypoxia will not only initiate plastic changes in the carotid bodies. In NTS neurons receiving afferent input from the carotid bodies of rats exposed to chronic intermittent hypoxia, dose-dependent currents induced by AMPA were increased whilst NMDA-activated currents were reduced (174). Reeves et al. (634) showed a chronic hypoxia-induced increase in the expression of the NR_1, NR_2A, and NR_2B NMDA receptor subtypes in the dorsocaudal brain stem. Another factor contributing to the centrally mediated HVR plasticity may be platelet-activator factor (PAF). In an attempt to gain insight into the role of this phospholipid, Reeves and Gozal (630) compared the effects of a chronic (30 day) intermittent hypoxic paradigm in mice deficient for the PAF receptor (PAFR) with those in their wild-type littermates. The results were somewhat contradictory. On the one hand, unlike wild-type mice, PAFR−/− mice failed to show an increase in normoxic ventilation after the challenge, indicating a role of PAF in chronic intermittent hypoxia-induced ventilatory LTF. On the other hand, over the period of 30 days, both genotypes showed an equally robust time-dependent increase in the response to sustained (20 min) hypoxia (630). Thus the precise role of PAF in the chronic intermittent hypoxia-induced augmentation of the HVR remains to be determined.
After exposure to chronic intermittent hypoxia, anesthetized rats showed a rise in blood pressure and an augmented preganglionic sympathetic nerve response to acute hypoxia (294). Conscious rats responded with an increase in the hypoxic response of the renal sympathetic nerve (339). Chronic intermittent hypoxia has a profound influence on the oxidative status of the brain cortex and leads to protein and nucleic acid oxidation, lipid peroxidation, and apoptosis, a picture that is mitigated in mice overexpressing Cu,Zn-SOD (857). ROS induced specific enzymatic changes in the brain stem of rats exposed to chronic intermittent hypoxia that eventually led to enhanced α-amidation of neuropeptides such as substance P and neuropeptide Y that thereby become biologically active (699). Many neuropeptides are operating in central neuronal networks, so similar mechanisms may also contribute to chronic intermittent hypoxia-induced changes in the HVR and sympathetic chemoreflex sensitivity.

In summary, in animals and across species, chronic intermittent hypoxia leads to an augmented episodic hypoxia-induced LTF at the level of carotid bodies, phrenic nerve, and ventilation. Carotid body sensitivity is increased and the HVR is enhanced. These adaptive changes are mediated by ROS-related HIF-1α stabilization and gene expression resulting in adaptations in the signaling-transduction cascade in the carotid bodies and plastic changes within the central respiratory neural network. As was also the case for chronic hypoxia, chronic intermittent hypoxia has different effects in neonates and adults. This is schematically shown in Figure 10.

3. Chronic intermittent hypoxia increases the HVR in humans

Protocols used in humans to produce chronic intermittent hypoxia vary widely in total duration (days to weeks), severity, and length of individual hypoxic exposures. In a minority of cases, isocapnia was maintained. Data we have collected from a number of recent studies are shown in Table 5. The paradigms employed do not exactly mimic the swings in PO2 and PCO2 that are encountered in sleep apnea syndrome wherein hypoxic episodes are shorter, 20 s on average, and accompanied by hypocapnia (242). Rather, they may represent time courses of prolonged hypoxic episodes that can occur in patients with chronic obstructive pulmonary disease (COPD) and/or severe heart failure. With only a few exceptions, paradigms of chronic intermittent hypoxia in humans result in an increase in the HVR, which can be accompanied by a rise in resting normoxic ventilation and drop in end-tidal CO2. In cases where isocapnic HVRs were measured at resting end-tidal CO2 levels (Table 5), the effects may even have been underestimated (postexposure end-tidal CO2 levels mostly being lower).

If it is accepted that the peripheral chemoreceptors mediate the initial hypoxia-induced rise in ventilation, enhancement of the acute HVR (8, 10, 11, 253, 484) is most probably due to an increase in carotid body sensitivity. A higher ventilation during hypoxia combined with an also higher arterial oxygen saturation is compatible with an increased slope of the ventilation-SaO2 relationship (e.g., Ref. 53). It is not easy, however, to assess the precise quantitative contribution of the carotid bodies to the augmented HVR: isocapnic progressive hypoxic tests lasting 5–9 min are associated with increases in CBF and central hypocapnia and may also be accompanied by a rapidly developing HVD (see sect. III). The fact that chronic intermittent hypoxia induces augmented cerebrovascular O2 and CO2 sensitivities should also be taken into account (8, 395).

The mechanisms of a chronic intermittent hypoxia-induced increase in carotid body sensitivity in humans remain elusive, but plastic changes as described for animals could well operate in humans also. Like in animals, the increase in HVR may depend on stimulus intensity, the number of deoxygenation/reoxygenation periods, and total exposure length. Exposing humans to a chronic inter-
**TABLE 5. Chronic intermittent hypoxia in humans**

<table>
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<tr>
<td>5 min isocapnic hypoxia (12% O₂) alternated by 5 min normoxia for 1 h, 12 days (protocol 1) versus 30 min isocapnic 12% O₂ for 12 days (protocol 2); interruption of protocols on days 6 and 7</td>
<td>Progressive isocapnic hypoxia to SaO₂ ~75 %; PeTCO₂ at resting values</td>
<td>↑ iHVR by 37% on day 12, recovery on day 17; no difference between both protocols; no changes in resting Vₐ and PeTCO₂</td>
<td>241</td>
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<tr>
<td>Daily exposure to a barometric pressure of 432 Torr for 1 h, 7 days</td>
<td>Step decrease in FIO₂ from 0.3 to 0.13; PeTCO₂ at level causing a hyperoxic Vₐ of 15 ml/min⁻¹·kg⁻¹</td>
<td>Max ↑ of iAHR, on day 5 and then gradually decreasing to control on day 12; isocapnic PeTCO₂ after hypoxia ~1.5 Torr lower</td>
<td>253</td>
</tr>
<tr>
<td>Daily three 6-min bouts of isocapnic hypoxia with PeTCO₂ 50-75 Torr interspersed with normoxia within 30 min, for 14 days</td>
<td>Progressive isocapnic hypoxia at eucapnic PeTCO₂</td>
<td>HVR, defined as the constant of the hyperbolic Vₐ-PaO₂ relationship, increases by 73 % no change in resting PeTCO₂</td>
<td>376</td>
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<tr>
<td>Decompression to 15.5 or 12.3 % O₂ for 1 h/day for 1 wk</td>
<td>Progressive isocapnic hypoxia to SaO₂ ~75 %; PeTCO₂ at resting values</td>
<td>↑ iHVR only after 12.3 % O₂ by ~25%, indicating an influence of the depth of hypoxia; no changes in resting PeTCO₂ and resting Vₐ</td>
<td>374</td>
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<tr>
<td>12% O₂ for 5 min, poikilocapnic, followed by 5 min normoxia, for 1 h, 10 days</td>
<td>Isocapnic progressive hypoxia to SaO₂ ~75 %; PeTCO₂ at resting values. Isocapnic progressive hypoxia to SaO₂ ~85% PeTCO₂ at resting values</td>
<td>No change in iHVR, resting PeTCO₂ somewhat lower after CIH</td>
<td>623</td>
</tr>
<tr>
<td>12% O₂ for 5 min, poikilocapnic, followed by 5 min normoxia, for 1 h, 10 days</td>
<td>Progressive isocapnic hypoxia to SaO₂ ~85% PeTCO₂ at resting values</td>
<td>No change in iHVR, neither in fit, nor in sedentary men; no change in resting PeTCO₂</td>
<td>700</td>
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<tr>
<td>12% O₂ for 5 min normoxia daily for 7 days SDIH versus 60 min poikilocapnic 12% O₂ daily, 7 LDIH in the same subjects</td>
<td>Isocapnic progressive hypoxia until SaO₂ ~80 % measured on 7 consecutive days; PeTCO₂ kept at resting values in all conditions</td>
<td>↑ iHVR by ~50% after both SDIH and LDIH, max effect reached after 3 days; CO₂ threshold (from modified rebreathing) decreased after both protocols</td>
<td>393</td>
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<tr>
<td>5 min 12% O₂, 5 min 21% O₂ for 90 min during 10–12 consecutive days</td>
<td>Poikilocapnic response to acute and sustained (20 min) hypoxia (12% O₂)</td>
<td>Acute, but not sustained pHVR response increased; no change in resting PeTCO₂</td>
<td>8</td>
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<tr>
<td>60 min of isocapnic hypoxia (SaO₂ ~80%) for 10 consecutive days</td>
<td>Progressive isocapnic hypoxia to SaO₂ ~75% and then maintained for 20 min; PeTCO₂ at resting values. iAHR determined from six descending PeTCO₂ steps with DEF technique; PeTCO₂ kept at ~1.5 Torr above resting</td>
<td>Sustained (20 min) iHVR doubles, no change in resting Vₐ and resting PeTCO₂</td>
<td>478</td>
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<tr>
<td>Sleeping for 5 consecutive nights with FIO₂ ~0.14</td>
<td>Isocapnic progressive hypoxia to SaO₂ ~80%</td>
<td>Mean iAHR more than doubles, baseline ventilation increased, resting PeTCO₂ fell by ~3.5 Torr</td>
<td>10, 11</td>
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<td>14 nights (9 h) of sustained SaO₂ ~84%</td>
<td>Isocapnic steps in hypoxia daily; modified rebreathing to determine thresholds and sensitivities both before and after isocapnic hypoxic studies</td>
<td>iHVR slope increases ~2.5-fold, increase in baseline ventilation; resting [paco₂] lower by 3.3 Torr</td>
<td>269</td>
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<tr>
<td>20 min of isocapnic (eucapnic) hypoxia (FIO₂ = 0.10) daily for 14 consecutive days</td>
<td>Isocapnic steps in hypoxia daily; modified rebreathing to determine thresholds and sensitivities both before and after isocapnic hypoxic studies</td>
<td>AHR but not sustained HVR increased; augmented AHR ascribed to decrease in peripheral CO₂ threshold because the threshold of the hypoxic, not the hyperoxic CO₂ rebreathing curve decreased. Sustained HVR not increased because of HVD, ascribed to an increase in peripheral CO₂ threshold</td>
<td>484</td>
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mittent hypoxia protocol with hypoxic episodes of 12% O₂ led to an increase in oxidative plasma biomarkers and a decrease in antioxidant capacity that were not observed with 15% O₂ (817). Katayama et al. (374) reported an increase in HVR by a protocol incorporating 12.3% O₂ but not 15.5%. Patients with obstructive sleep apnea show an increase in plasma biomarkers of oxidative stress and ROS production in leukocytes (202, 365). A recent study in healthy young subjects showed that after 4 days of intermittent hypoxia, the acute ventilatory response to hypoxia increased, which was correlated with an augmented production of ROS (605).

Long-term chronic intermittent hypoxia in humans has pathophysiological consequences that are recently reviewed elsewhere (242). Briefly, it leads to an increase in (muscle nerve) sympathetic activity, impaired baroreflex, increased production of ET-1 by enhanced shear stress, decreased NO bioavailability, impaired vasodilation, and hypertension. Increased production of ROS causes upregulation of genes encoding factors that elicit inflammation, platelet aggregation, and atherosclerosis (242). Chronic intermittent hypoxia as experienced by patients with a sleep apnea syndrome will tend to increase their HVR. An increase in chemoreflex sensitivity elicits higher sympathetic activity and eventually results in hypertension in many of these patients (372, 537, 548).

### 5. Different effects of chronic hypoxia and chronic intermittent hypoxia

The effects of chronic hypoxia and chronic intermittent hypoxia may be difficult to compare. For example, differences in the duration and severity of individual hypoxic episodes, total exposure time to hypoxia, and the large variation in cycle number of intermittent hypoxic paradigms render it hard to define one single effect chronic intermittent hypoxia. Some protocols may comprise both chronic hypoxia and chronic intermittent hypoxia or repetitive cycles with different lengths. Protocols with long uninterrupted exposures, e.g., during long nights spent in hypoxia (e.g., Refs. 11, 269) lead to increased carotid body sensitivity and periodic breathing and an ensuing additional, chronic intermittent (hypercapnic) hypoxia superimposed on the experimental one. This may be a confounding factor in studying the effects of “chronic hypoxia” during long stays at high altitude.
The effect of chronic intermittent hypoxia clearly depends on the pattern of the hypoxic exposures. For example, rats exposed to chronic intermittent hypoxia with long uninterrupted hypoxic episodes did not manifest an augmented carotid body sensitivity, in contrast to those challenged with hypoxia of equal total duration but with many oxygenationreoxygenation cycles; the former animals may have experienced a milder oxidative stress compared with the latter (598). The production of ROS will widely vary not only with the length and severity of the hypoxic episodes but also on the number of reoxygenation periods. A single bout of hypoxia and reoxygenation produce ROS, and this effect is amplified by repetitive hypoxia/reoxygenation (857, 867).

Some of the reported different consequences of chronic hypoxia and chronic intermittent hypoxia can be listed as follows. 1) In contrast to chronic hypoxia, a specific chronic intermittent paradigm did not lead to morphological changes in the carotid bodies (585, 596). 2) In PC12 cells, the time course and magnitude of HIF-1α expression differed between the two paradigms: chronic intermittent hypoxia was more potent in increasing HIF-1α protein, and the elevated levels were sustained for longer periods of time (references in Ref. 536). 3) Another study reported that in contrast to chronic hypoxia, chronic intermittent hypoxia did not enhance HIF-1α mRNA in the rat carotid body, while both paradigms augmented the expression of HIF-2α and HIF-3α (434). 4) In the dorsocaudal brain stem of the rat, chronic intermittent hypoxia was associated with an increase in the expression of NR2A and NR2B subunits of the NMDA receptor that did not occur in chronic hypoxia (634). The authors of this study related this to the less pronounced presence of HVD after the chronic intermittent compared with the chronic protocol (634). 5) As discussed in section VGI, in the rat, chronic intermittent but not chronic hypoxia augmented the magnitude of LTF (597).

Using a systems biology approach, Wu et al. (850) studied the expression of genes, sets of genes, and genetic networks and found different temporal expression patterns in chronic- and chronic intermittent hypoxia in the lungs of male rats. The gene encoding the estrogen receptor 1 (Esr-1) was unmasked as an important regulator of the response to chronic intermittent but not to chronic hypoxia. Increased expression of Esr-1 also resulted in upregulation of >200 target genes of the ESR-1 protein, for example, the P2X2 purinergic receptor. In addition, ESR-1 closely interacted with gene networks that already were known to play key roles in hypoxia, for example, a hub node around HIF-1β and vascular endothelial growth factor (VEGF). Chronic hypoxia acutely induced a different set of genes that were overrepresented by those involved in the immune response. The network activated by chronic hypoxia included the hub nodes with vascular endothelial growth factor, nuclear factor-κB and transforming growth factor-β, all of which are known to play a role in pulmonary hypertension (850). This interesting approach awaits application in the carotid bodies.

VI. MEASURING, EXPRESSING, AND MODELING THE HYPOXIC VENTILATORY RESPONSE

A. Measuring the HVR

Techniques used to measure the human HVR can be divided into five groups: 1) steady-state techniques involving fixed inspired O2 and CO2 concentrations, 2) single or double breath tests involving transient hypoxia or hyperoxia for no more than a few breaths, 3) progressive hypoxia tests involving the rebreathing of a gas mixture from a rebreathing bag or circuit with CO2 absorption allowing control of end-tidal CO2, 4) step hypoxic tests in which rapid decreases in end-tidal O2 are performed while maintaining end-tidal CO2 constant, and 5) via measurement of isoxic hypoxic and hyperoxic ventilatory CO2 responses.

The HVR obtained with steady-state hypoxic tests (test 1) is a mixture of the acute effects of hypoxia and its central depressant effects. Hence, the response is “contaminated” by HVD. These tests were applied in a time and age in which there was little knowledge on HVD. Consequently, steady-state tests are considered outdated (871). Depjouis et al. (177) developed the single breath test (test 2) in which after several control breaths the subject inhales one vital capacity of a test gas (100% O2 or 100% N2 with or without the addition of CO2). The change in ventilation during the next few breaths is a measure of hypoxic sensitivity. While this test is not contaminated by HVD, it does not allow the development of the full HVR, and isocapnia is not maintained during the test. Furthermore, to avoid random effects from the background variation in ventilation, the test has to be repeated frequently (411). Finally, quantification of the small and short-lived change in ventilation is difficult (628), and therefore, the test should be just considered a qualitative measure of peripheral chemoreceptor activity. At present, most popular tests are those that use progressive (test 3) and step hypoxia (test 4) and those that measure the HVR via the V1-CO2 responses at hyperoxia and hypoxia (test 5).

1. Progressive hypoxic tests

Over the years, various progressive hypoxic tests have been designed (143, 272, 411, 458, 485, 626–628,
Techniques differ in depth of hypoxia, steepness of the hypoxic ramp (and linked to this the duration of the test), and the technique to keep end-tidal CO\textsubscript{2} constant. Weil et al. (833) published their method in 1970: subjects breathed sequentially three gas mixtures for 3–5 min (room air, 10 and 8.5% O\textsubscript{2} in N\textsubscript{2}) causing a slow (15 min) progressive isocapnic decline in alveolar PO\textsubscript{2} to ~40 Torr. Isocapnia was maintained by adding CO\textsubscript{2} to the inspiratory limb of the breathing circuit. Godfrey et al. (272) adapted the CO\textsubscript{2} rebreathing technique developed by Read (624) in such a way that hypoxia developed progressively over 5–8 min. The subjects rebreathed from a circuit that included a CO\textsubscript{2} absorber allowing the maintenance of isocapnia. The initial gas mixture contains 25–30% O\textsubscript{2} in N\textsubscript{2}, and as the subjects consumes oxygen, the oxygen concentration of the bag decreases. During the course of rebreathing, arterial PO\textsubscript{2} drops from 130 to ~30 Torr. Rebuck et al. (627) and later Rebuck and Campbell (626) described a rebreathing technique similar to that of Godfrey et al. (272). The “Rebuck” approach involves rebreathing from a 6-liter bag containing a gas mixture of 7% CO\textsubscript{2} and 22–24% O\textsubscript{2} in N\textsubscript{2}. The obvious problem with this technique is that the ensuing HVR is due not only to the progressive decline in PO\textsubscript{2} but also to the step increase in PCO\textsubscript{2} (658). In a modified approach, the high PCO\textsubscript{2} level is kept constant for 6 min before beginning to rebreathe from the bag (485). As pointed out by Robbins and Zhang (658), the later description of the modified “Rebuck rebreathing technique” by Rebuck and Slutsky (628) does not make it clear that the original technique is seriously flawed. See Figure 11A for a description of the apparatus used by Rebuck and colleagues. It is important to be aware that rebreathing methods have the potential for systematic errors due to contamination by HVD when tests last longer than 5 min (411, 612).

2. Step hypoxic tests

A sophisticated method to obtain ventilatory responses to steps into and out of hypoxia is by using the computer-controlled DEF technique, developed by Swan- son and Bellville in the late 1960s and early 1970s in Stanford, California (56, 747, 748). Originally DEF was used to measure the \( V_{F} \cdot \text{CO}_2 \) response and quantify the contribution of the central and peripheral chemoreflex loops to total ventilation (56). Not much later, it was adopted by several research teams to study step and sinusoidal hypoxic responses (176, 655, 822). DEF (see Fig. 11B) allows manipulation of the inspired PO\textsubscript{2} and PCO\textsubscript{2} to induce specific waveforms in end-tidal gas concentrations independent of the ventilatory response and venous return. Feedback/forward loop algorithms are applied to anticipate and compensate for the respiratory response to a desired end-tidal gas concentration on a breath-to-breath basis. The control of the end-tidal gas concentrations with DEF is good, with standard deviations of the end-tidal concentrations over time <0.6 Torr (161). The main advantage of DEF is its ability to obtain an open-loop estimation of ventilatory responses within a short period of time, something that is impossible under closed-loop conditions.

The technique has the disadvantage that relatively complex apparatus is required. As a result, less sophisticated techniques are utilized more frequently using containers with preset hypoxic mixtures and/or adaptations of the inspired oxygen concentrations by hand to obtain the desired end-tidal values (204, 370, 683, 705). Often isocapnia is maintained by using a rebreathing circuit (683), otherwise by adding CO\textsubscript{2} to the inspiratory limb of the apparatus (204).

One variant of the step hypoxic test is the application of randomly modulated sequences in hypoxia and hypercapnia (863). This computer-driven technique requires mathematical modeling to obtain an indication of the magnitude of the HVR and its interaction with CO\textsubscript{2}. While of great value in the characterization of the ventilatory control system (see also sect. viC), it is of lesser practical applicability.

3. Measuring HVR via the \( V_{F} \cdot \text{CO}_2 \) response

Various authors derive the HVR from isoxic hypoxic and hyperoxic \( V_{F} \cdot \text{CO}_2 \) response curves (195, 349, 411, 547, 628, 720). Most frequent studied PO\textsubscript{2} values for hyperoxia are 150–250 Torr and for hypoxia are 40–50 Torr. Severinghaus defines the hypoxic response as the increase in ventilation at an interpolated end-tidal CO\textsubscript{2} going from hypoxia to hypoxia (\( \Delta V_{40} \) when a 40 Torr hypoxic level is chosen, Ref. 683). Others use the change in slope going from hyperoxia to hypoxia (\( \Delta S \)) or the ratio of the slopes (\( S_{40}/S_{200} \)) as well as the change in the position of the response curves (195, 628). When the isoxic \( V_{F} \cdot \text{CO}_2 \) response curve is assessed using steady-state methods at a hypoxic background, an appreciable effect of HVD on the response occurs. To overcome this problem, some authors use the (modified) CO\textsubscript{2} rebreathing procedure according to Read (624). Note, however, that the Read-rebreathing technique, although relatively simple in performance, contains inherent difficulties that are not always appreciated (155). For example, abolishing the mixed venous-to-arterial PCO\textsubscript{2} gradient, one of the key features of the Read-rebreathing technique (624), is not enough to reduce the brain tissue-arterial PCO\textsubscript{2} gradient sufficiently to zero to cause equilibration between arterial and brain tissue PCO\textsubscript{2} (155).

4. The choice of method of measuring HVR

a) The choice of test end-tidal PCO\textsubscript{2}. There is no consensus on defining the test end-tidal or arterial PCO\textsubscript{2} (696). Some investigators raise the end-tidal value ar-
FIG. 11. Experimental set-up to measure the HVR. A: setup used by Rebuck and Slutsky to measure the ventilatory response to hypoxia. The DC pump draws gas from the bag through the soda lime and returns the gas to the end of the bag (arrows). [Adapted from Rebuck and Slutsky (628).] B: experimental setup used in Leiden to apply dynamic end-tidal forcing of oxygen and carbon dioxide. There are two computer subunits, one to apply control signals to the mass flow controllers, which sets the desired inspiration oxygen and carbon dioxide fractions, and one to collect the end-tidal data.
bitrarily (e.g., to 45 Torr), and others use a fixed increase relative to baseline end-tidal CO₂ (e.g., +5 Torr above resting) or a value set to achieve equal background central drive throughout the hypoxic experiment (for example, by using an end-tidal CO₂ value at which ventilation in hyperoxia equals 4 l/min or 140 ml·min⁻¹·kg⁻¹), presumably to prevent a possible confounding influence of central-peripheral interaction (195, 696, 720). The method of choice of the end-tidal CO₂ is of importance when studying the effect of specific interventions, such as high altitude, acid-base alterations, or pharmacological agents, on the HVR. An insightful and acceptable alternative to the measurement of HVR at a fixed end-tidal PCO₂ is the assessment of HVR at two to three P CO₂ values (Fig. 12). Alternatives could be ΔS or S₁⁴0/S₂⁵⁰.

B) THE CHOICE OF BASELINE END-TIDAL PO₂. When studying HVR, the baseline oxygen level chosen is sometimes hyperoxic (end-tidal PO₂ 150–250 Torr; Refs. 195, 696). This is done to “silence the peripheral chemoreflex response to CO₂” (195). Whether this is achieved at mild hyperoxia is doubtful. Data from Pedersen et al. (591) indicate that at 200 Torr, the fast peripheral component of the V₁-CO₂ response remains present in humans (mean peripheral component was 27% of total CO₂ sensitivity). Even at much “deeper” levels of hyperoxia (end-tidal PO₂ >500 Torr), the human carotid bodies remain active with an average 13% contribution of the peripheral component to total CO₂ sensitivity (156). Furthermore, high oxygen levels affect central respiratory drive via the Haldane effect and a reduction of brain blood flow (this effect is independent of any CO₂ inhalation), causing a rise in brain tissue PCO₂ (156). High oxygen may also cause the formation of excitatory substances in the brain (e.g., NO, glutamate, ROS). Although these components seem of lesser importance during mild hyperoxia (140–250 Torr), we cannot exclude some excitatory effect on breathing from even brief increases in O₂ (see Fig. 13; Ref. 613). With all of the above taken into account, our first choice would be to assess the HVR using a normoxic baseline level. The choice of a hyperoxic baseline level has little or no advantage, and in addition, from a physiological view point, hyperoxia does not represent a realistic ambient state.

C) A PROPOSAL FOR MEASUREMENT OF HVR. Over the years, Severinghaus and others made several attempts to reach consensus on the HVR methods (131, 195, 696, 697). Consensus is required to overcome “lack of comparability between results obtained by various groups due to their variety in methods, definitions, choice of isocapnic P CO₂ and duration of hypoxia” (696). In the so-called “proposed Lake Louise HVR Methods,” Severinghaus (696) proposes three tests (Table 6): 1) an isocapnic HVR (iHVR) to determine the hypoxic sensitivity of the carotid bodies lasting no longer than 5 min and 2) a poikilocapnic HVR (pHVR) lasting 20 min to predict the response to high altitude. In both tests, baseline (prehypoxia) conditions are hyperoxic (150 < end-tidal PO₂ < 250 Torr). 3) The iHVR may be prolonged to get an indication of the isocapnic HVD. Duffin (195) proposes three tests (Table 6): 1) the assessment of peripheral chemoreflex responsiveness by measurement of the ventilatory response to CO₂ at hyperoxia (150 Torr) and hypoxia (40 Torr) preferentially performed by using the modified Read-rebreathing test (or alternatively performing 5-min isocapnic steps from hyperoxia to hypoxia); 2) to get an indication of HVD
the first procedure is repeated before and after 20-min
of hypoxia (or alternatively performing a 20-min iso-
capnic hypoxic response; and 3) a 20-min poikilocap-
nic step hypoxic response is performed to mimic the
response to environmental hypoxia.

Our proposal is a modification of the methods out-
lined by Severinghaus and Duffin (Table 6: the “Leiden”
proposal). Similar to the other proposals, we suggest
performing three distinct tests. In test 1, 5-min step re-
sponses into hypoxia (arterial P O₂ 45–50 Torr) are per-
formed at three different arterial PCO₂ levels (e.g., /H₁₁₀₀₁
2, /H₁₁₀₀₁
4, and /H₁₁₀₀₁
8 Torr above resting). However, in contrast to the
previous proposals, baseline oxygen levels are normoxic
(arterial PO₂ = 100 Torr). One is now able to calculate the
acute hypoxic response (i.e., hypoxic sensitivity), and
since the responses are obtained at various PCO₂ levels,
one gets a full characterization of O₂-CO₂ interaction, a
phenomenon most likely originating at the peripheral che-
moreceptors (see also sect. vD; Fig. 12). Tests 2 and 3 are
similar to the ones proposed by Severinghaus but with
normoxic rather than hyperoxic baseline states to prevent
any excitatory effect of even mild hyperoxia. A transition
from hyperoxia to hypoxia will change central ventilatory
drive more than the transition from normoxia to hypoxia
(see sect. viA 4b). This will affect the position of the V˙I-CO₂
response causing a leftward shift (Fig. 13). Of equal im-
portance is the fact that hyperoxia does not silence the
peripheral chemoreceptors. Furthermore, we refrain from
obtaining V˙I-CO₂ responses curves at different P O₂ levels
as proposed by Duffin (195) as ion channel and neuro-
transmitter responses to low oxygen and high PCO₂/low
pH stimulation at the carotid bodies may differ and, as is
also important, arterial PO₂ is a separate stimulus at the
peripheral chemoreceptors (277). Furthermore, by per-
forming step hypoxic tests, one may decide to use peak
hypoxic ventilation rather than 5 min of hypoxia as hy-
poxic measurement point. This is especially important
when the temporal profile of the response indicates that
HVD is apparent at times <5 min of hypoxia (see also Fig.
13 for an example).

Fig. 13. Measuring the hypoxic ventilatory response at three constant levels of end-tidal P CO₂. A: isocapnic ventilatory responses to hypoxia
and hyperoxia at three arterial P CO₂ levels obtained in a healthy 28-year-old female volunteer. Normoxic, hyperoxic (arterial P O₂ = 140 mmHg), and
hypoxic (arterial P O₂ = 45 mmHg) data points are given. The data points form post hoc steady-state V˙I-CO₂ response curves in normoxia, hyperoxia,
and acute hypoxia (lines through the data points derived from linear regression). The left shift of the hypoxic V˙I-CO₂ response curve relative to
the normoxic curve is due to the central stimulatory effects from even mild hyperoxia. Consequently, using the hypoxic data as a measure of the
isocapnic HVR (see Table 1) makes data interpretation difficult if not impossible. B: the data in these graphs are obtained by using a small adaptation
to the test 1 of the “Leiden proposal” of Table 6. The top graph shows the imposed end-tidal fractions of O₂ (continuous line) and CO₂ (dotted line).
In between the normoxic and hypoxic exposures, a 7-min isocapnic hyperoxic exposure (P O₂ = 140 mmHg, measured in arterial blood) was added.
The middle graph shows the measured arterial O₂-Hb saturation using pulse-oximetry. The bottom graph shows the measured inspired ventilation.
Each data point is one breath.
| Test 1: isocapnic HVR (iHVR) to assess the hypoxic sensitivity of the carotid bodies |
| Baseline oxygen level | 150 < end-tidal P_{O_2} < 250 Torr for 10 min |
| Hypoxic test | Reduce the inspired O_2 level such that the SPO_2 is rapidly lowered and maintained at 80% (range 75–85%) for 5 min |
| P_{CO_2} | Keep the end-tidal P_{CO_2} constant at a level causing hyperoxic ventilation to rise to a standard, e.g., 140 ml·min^{-1}·kg^{-1} |
| Expression of response | iHVR = ∆V_{I}/∆SPO_2 where ∆V_{I} = the increase in V_{I} from baseline to the 5th min of hypoxia |

| Test 2: measuring the isocapnic hypoxic ventilatory decline (iHVD) |
| Hypoxic test | Expand test 1 to 20 min of isocapnic hypoxia |
| Expression of response | % fall in ∆V_{I} from the 5th to the 20th min |

| Test 3: poikilocapnic HVR (pHVR) to predict the HVR at altitude |
| Baseline oxygen level | 150 < end-tidal P_{O_2} < 250 Torr for 10 min |
| Hypoxic test | Inspiration of an O_2/N_2 gas mixture (e.g., 12% O_2) to reduce SPO_2 to 80% for 20 min |
| Expression of response | pHPR = ∆P_{ETCO_2}/∆SPO_2 |

*An alternative is the measurement of a three-point steady-state V_{I}-CO_2 responses performed at hyperoxia and hypoxia. **An alternative test is an isocapnic 20-min step hypoxic test as suggested by Severinghaus (696). iHVR, isocapnic hypoxic ventilatory response; pHVR, poikilocapnic hypoxic ventilatory response; pHPR, response of the end-tidal P_{CO_2} to poikilocapnic hypoxia; SPO_2, arterial Hb oxygen saturation as determined by pulse oximetry; P_{ETCO_2}, end-tidal P_{CO_2}; V_{I}, inspired minute ventilation.
B. Expressing the Steady-State HVR

Mathematical expressions of the HVR have to take into account the fact that the steady-state relationships between ventilation and arterial and end-tidal PO2 are nonlinear. The HVR has been quantified using hyperbolic and exponential expressions (411, 697), and there is no physiological reason to select one over the other.

The hyperbolic expression (457, 697, 833)

\[ \dot{V}_I = \dot{V}_0 + A/(PO2 - C) \]  

(1)

where \( \dot{V}_0 \) is the horizontal asymptote in \( \dot{V}_I \) for infinite high PO2 values, A is the hypoxic sensitivity, and C is the vertical asymptote in PO2 for infinite high \( \dot{V}_I \) values. In most studies, the asymptote C is fixed to 32 Torr, which causes a bias in the estimation of the parameter A (214). Bayesian estimation schemes using soft constraints on C are advocated to improve the estimation of parameter A (214). The hyperbolic expression indicates that the reciprocal of ventilation versus PO2 is a linear relationship, an observation that has been established experimentally (272). The exponential expression (58, 176, 411, 697)

\[ \dot{V}_I = \dot{V}_0 + G \cdot \exp(-D \cdot PO2) \]  

(2)

where \( \dot{V}_0 \) is \( \dot{V}_I \) for infinite high PO2 values, G is the hypoxic sensitivity, and D is a shape parameter. The shape parameter is often fixed to a specified value (e.g., to 0.29%−1; Ref. 824). This is done when the O2 input is not rich enough to provide an estimation of D (e.g., when employing single steps into hypoxia). This value of D has been estimated by fitting an exponential through the function 100 − Z, where Z is the saturation calculated from the Hill model of the oxygen dissociation curve (824). The use of an exponential model is equivalent to the empirically linear relation of log d\( \dot{V}_I \) to PO2, as used by Kronenberg et al. (411).

Since there is an empirical linear relationship between \( \dot{V}_I \) and blood oxygen desaturation (626, 628), a more simple approach would be to express ventilation as a linear function of oxygen saturation

\[ \dot{V}_I = \dot{V}_0 + S/([SpO2(0) - SpO2]) \]  

(3)

where \( \dot{V}_0 \) and SpO2(0) are prehypoxic ventilation and saturation, respectively, and S is the hypoxic sensitivity (units, L·min−1·%−1). There are several reasons, however, why saturation may not be the most appropriate tool to express the HVR. Oxygen saturation is not the stimulus to the oxygen sensors at the carotid bodies (e.g., Ref. 313). The \( \dot{V}_I \)-SpO2 relationship is not under all circumstances and protocols linear (159, 336, 384). And, most importantly, a change in the position of the oxygen dissociation curve (reflected in a change in P50) creates an artifact with an incorrect calculation of HVR (613). For example, a metabolic acidosis increases P50 and changes the value of \( \Delta V_I/\Delta SpO2 \) without any change in the relationship between \( \dot{V}_I \) and PO2 and hence of the HVR. And from a technical point of view, it is our experience that at low saturation levels (< 80%), measurements derived from pulse oximetry are a less reliable measure of the actual arterial HbO2 saturation.

In the cat, it has been demonstrated that in the hypoxic range, ventilation is a linear function of \( \log PaO2 \), while hypoxic sensitivity, defined as the ratio \( \Delta V_I/\log PaO2 \), is linearly related to the arterial H+ concentration (650). Recently, we found that in humans relative changes in hypoxic sensitivity induced by alterations in arterial PCO2 are the same regardless of how the sensitivity was defined, either as \( \Delta V_I/\log PaO2 \) or as \( \Delta V_I/\Delta SpO2 \) (775). As in cats, humans also showed a linear relationship between hypoxic sensitivity (defined as \( \Delta V_I/\log PaO2 \)) and arterial H+ concentration (see Fig. 7; Ref. 775). Future studies in humans are warranted to see if, as in the cat, ventilation is a linear function of \( \log PaO2 \) in the hypoxic range. If this would appear to be the case, then we would suggest the use of the expression \( \Delta V_I/\log PaO2 \) to estimate hypoxic sensitivity, with end-tidal O2 but preferentially arterial PO2 as input. It is a simple approach, alike to the use of oxygen saturation but without the confounders that are inherently present in the use of SpO2. When performing multiple steps into hypoxia (e.g., at three different background PCO2 levels), it further enables the description of O2-CO2 interaction (see Figs. 7 and 12) and the effects that drugs may have on this important feature of the peripheral chemoreceptors (such as abolishment of O2-CO2 interaction by inhalational anesthetics).

C. Modeling the HVR

Several models allow the estimation of physiologically relevant parameters (hypoxic sensitivities/gains and time constants) from single experimental data sets. Initial mathematical models of the ventilatory response to iso-capnic hypoxia consisted of two additive components: an initial stimulating component arising from the carotid bodies and a second, slower component that causes a decline in ventilation (176, 824). These models were unable to fit human responses into and out of hypoxia simultaneously due to the asymmetry in the magnitude of the fast on- and off-transients (824). The model proposed by DeGoede et al. (176) is an exponential expression, in which the two components differ with respect to their dynamics (expressed by a time constant or \( \tau \), gain values, and input latencies. One of the first human studies (824) yielded the following parameter values: fast component: \( \tau = 6 \) s, gain of response into hypoxia 50 l/min, gain of response out of hypoxia 30 l/min; slow component: \( \tau = 4 \)
min, gain of response into hypoxia −40 l/min (i.e., HVD), gain of response out of hypoxia −20 l/min (relief of HVD). The asymmetry in the gain values suggests modulation of the peripheral component during sustained hypoxia as the cause of HVD (cf. Ref. 162).

Khamnei and Robbins (382) examined how HVD is best incorporated in models of the HVR. They concluded that modulation of the peripheral chemoreflex gain during sustained hypoxia consistently describes the human data in contrast to models in which hypoxic depression is modeled as an additive component or as a modulator of central or total CO₂ sensitivity (382). In a quantitative modeling study (by assuming that the peripheral gain term is a linear function of arterial oxygen saturation), this was confirmed and moreover allowed (the asymmetry of) the ventilatory response into and out of hypoxia to be analyzed simultaneously (577). However, in some subjects, the description of the response out of hypoxia (the off-transient) remained inadequate. Therefore, to optimize the model, a further model expansion was tested in which HVD was modeled by modulation of the peripheral chemoreflex gain (577) combined with a component that is independent of the peripheral chemoreflex loop (445). The extended model yielded significantly better fits in just two of six subjects. Both had a chemoreflex-independent HVD component >50%. This contrasts with a value of ~10% in the four other subjects examined. Liang et al. (445) concluded that in some subjects, but not others, there might be a component of HVD that is independent of the peripheral chemoreflex sensitivity.

Some studies, human and animal, indicate that the fast hyperventilatory response to isocapnic hypoxia is best described by a fast and a slower component (58, 198, 384, 550) with time constants for the fast and slow components of 2–3 s and 70–100 s, respectively (58, 550). Possibly, the slow peripheral component is a manifestation of central neuronal dynamics, equivalent to the slow component observed when the carotid sinus nerve is electrically stimulated (211). This then suggests that the HVR must be modeled by at least three components, two of peripheral origin, one of which (or both?) is modulated by central or total CO₂ sensitivity yielding significant values for a trend in the order of 70 ml/min² (156).

VII. COMMENTS AND FUTURE CHALLENGES

At sea level, hypoxia is a relatively rare event in healthy individuals, except in utero and perhaps during a neonatal period in which irregular breathing is not uncommon, or during sleep when wakefulness drive is absent and one may sometimes discontinue breathing. When healthy individuals are exposed to inspired oxygen tensions lower or higher than normal, for example, at high altitude or in an experimental setting, the respiratory system responds in a way that critically depends on the stage of maturation (neonates show different responses than adults), the physiological background condition (e.g., CO₂ and exercise increase the HVR), the intensity of the stimulus, and, last but not least, the time pattern and duration of the exposure. Acute, chronic, and episodic hypoxia with or without a chronic character all have different effects on ventilatory control. Both in neonates and adults, the respiratory system displays remarkable plasticity when exposed to intermittent and/or chronic hypoxic paradigms that lead to changes in HVR phenotypes. Animal studies show that when hypoxic stimulus paradigms (or environmental factors such as nicotine exposure or maternal separation) are imposed during a neonatal critical time window, plastic changes persist into adulthood and are difficult to reverse, if at all. An important challenge for future research is to examine whether the underlying mechanisms that are responsible for these changes also apply in humans.

An important circumstance that mediates plasticity-induced respiratory changes in rodents is neonatal oxidative stress. Rats exposed to chronic intermittent hypoxia develop an augmented carotid body sensitivity and an increased HVR, changes that persist into adulthood (585; note, however, that as explained in section II, the increase in HVR is not without controversy). If translatable to human newborns, this raises a number of clinically relevant issues. Newborn babies, especially when premature, display irregular breathing that can be accompanied by frequent apneic events. Does this disturb a normal maturation of the HVR in premature babies, and might this lead to sensitization of the carotid bodies thereby destabilizing breathing just like in animals? Further destabilizing breathing might lead to a higher frequency of apneic events, an increase in the number of deoxygenation/
re-oxygenation cycles, and finally to an increased production of ROS that would further aggravate the oxidative stress (in neonatal rats the augmented carotid body sensitivity is a ROS-related phenomenon; Ref. 586). And can possible changes in the HVR be reversed at adult or possibly at earlier age? An important difference between newborn and adult rats is the much higher sensitivity to chronic intermittent hypoxia of the former with respect to the effects on carotid body sensitization, morphological changes (e.g., hyperplasia), and reversibility (see Fig. 10; Ref. 585).

In neonatal animals, within a critical time window of plasticity in the period after birth, chronic hypoxia impairs carotid body maturation and O₂ sensitivity, causes degenerative changes in glomus cells and sinus nerve afferents, and reduces both the CNS gain and the HVR (Fig. 9). The fact that at the level of ventilation the diminished response to hypoxia persists into adulthood indicates that at least some of these adaptations are permanent and perhaps difficult to reverse. If similar mechanisms are operating in humans, the question arises as to whether chronic hypoxia in newborn babies, when untreated, may have similar deleterious effects that ultimately may lead to a decreased ability to respond to an asphyxic stimulus, long-lasting exposures to hypoxia, and failure to autoresuscitate. However, at least in the western world, the clinical relevance of this issue may be limited because when newborn babies are hypoxic, for example, due to hypoventilation, ventilation perfusion mismatch, intrapulmonary or cardiac shunts, anemia, etc., they will be rapidly treated with oxygen. On the other hand however, apart from the well-known detrimental side effects of oxygen (for example, retinopathy; see Ref. 816 for further references), a permanently reduced HVR (from chronic oxygen therapy) in these children that persists into adulthood cannot be excluded. In rats, neonatal chronic hypoxia can cause a reduced HVR at adult age depending on the level of hypoxia and the duration of the neonatal exposure (41, 71, 72).

An important point to consider when putting data from neonatal animals into the context of newborn babies is relative maturity at birth. If 1- to 5-day-old rats resemble preterm babies in their development of the HVR (73), the findings from this species may be less relevant for term babies. This, however, does not obviate the need to focus attention on possible adverse effects of neonatal oxidative stress (both hypoxia and hyperoxia) on the HVR in both preterm and term babies.

In adult healthy humans, chronic intermittent hypoxia results in an augmented HVR, presumably related to oxidative stress (increased production of ROS; Refs. 374, 605, 817). Studies in humans on the effects of chronic intermittent hypoxia are often aimed at mimicking hypoxic episodes that occur in patients with obstructive sleep apnea (OSA). This chronic disease is associated with an enhanced sympathetic activity, systemic hypertension, and an augmented HVR. Similar to animals exposed to chronic intermittent hypoxia, OSA patients display increased production of ROS (202, 365). The increases in both systemic blood pressure and HVR can be reversed with nasal continuous positive airway pressure treatment (790, 791). To mimic the complex events that occur in OSA in a laboratory setting may be elusive in the sense that (awake) healthy subjects do not display the increase in resting tone of upper airway dilator muscles that in OSA patients serves to compensate for a decreased patency of the upper airways. This may well be one of the reasons why it is difficult if not impossible to evoke LTF of upper airway dilator muscles in healthy sleeping subjects but less so in snorers with upper airway flow limitations (21). Apart from the absence of obstructive apneas, an additional limitation of studies in awake healthy subjects is the lack of arousals that in sleeping OSA patients may play a crucial (albeit not clearly defined) role and the absence of the influences of sleep itself on the control of breathing. And finally, in OSA patients, apneic events followed by hyperventilation are associated with alternating episodes of low and high PCO₂, which makes the stimulus paradigm fundamentally different from that in most laboratory studies in which the hypoxic episodes are hypocapnic or at best isocapnic (Table 5). On the other hand, studies in animals and healthy humans are of considerable interest because they can uncover mechanisms by which chronic intermittent hypoxia by itself can lead to maladaptations such as inflammation, endothelial dysfunction, atherosclerosis, and other cardiovascular pathology.

From studies over the last decade, the picture emerges that oxygen sensing by the carotid bodies occurs via several parallel pathways. The key role of potassium channel species is well established, but identification of individual members involved is complicated by species- and even strain-related differences in potassium channel regulation by hypoxia. AMP-activated protein kinase (AMPK), an enzyme considered to play a key role in cellular metabolism, has been identified as a link between hypoxia-induced changes in mitochondrial metabolism and potassium channel activity. Mice deficient in the α₂ subunit of AMPK show an attenuated frequency response to hypoxia in vivo (222). A possible role of AMPK in glucose sensing by the carotid bodies, in the adaptation to high altitude, as well as in exercise remain interesting subjects for further study. To uncover the role of the numerous individual type I cell neurotransmitters in the integrated response of the carotid body to hypoxia is another challenging issue.

A fascinating example of respiratory plasticity at adult age becomes manifest during adaptation to chronic hypoxia. In fact, all elements in the carotid bodies and CNS known to participate in the hypoxia signaling trans-
duction cascade undergo plastic changes during chronic hypoxia. How does the organism manage to orchestrate so many changes (recalibrations) at the same time without losing control over system stability? One of the master regulators now identified is HIF-1, a transcription factor that regulates the expression of numerous genes encoding elements involved in improving tissue oxygenation (HVR, erythropoiesis, angiogenesis, etc.) and in metabolic adaptations (increased ATP supply from glycolysis, optimization, and reduction of oxidative phosphorylation). Systems biology approaches are currently used to map individual genes, untangle genetic networks, and identify their hubs (441, 850). Systems biology also offers an important avenue to discover cross-talk between hypoxia and other key signaling mechanisms. For example, it has been shown that angiogenesis in hypoxia is induced by cross-talk with notch signaling (441); notch receptors are highly conserved transmembrane proteins thought to be involved in cell-to-cell communication and cell differentiation (references in Ref. 441). Similar mechanisms may operate in the carotid bodies, but efforts to examine this have not yet been undertaken.

How hypoxia influences the stability and activity of transcription factors is also focus of current interest. The fact that transcription factors may also undergo posttranscriptional modification independent of hypoxia is an interesting but complicating factor in the interpretation of these studies because hypoxia-independent pathways may then autonomously influence the expression of genes involved in the adaptation of the HVR to chronic conditions. For example, HIF-1α can be stabilized by NO-mediated S-nitrosylation and by heat shock protein 90, and both processes are hypoxia independent (692). With regard to HIF-1α, targeting its stability independent of the Po2 may appear to open new treatment options of HIF-1-related pathology. For example, an interesting recent observation in humans is the reversal of hypoxia-induced pulmonary hypertension by iron supplementation (713; further references in Refs. 441, 692).

Animal studies indicate an important effect of genotype on the HVR phenotype (i.e., the HVR magnitude). With the relatively large effect of environment and prefrontal cortex on the magnitude and variability of HVR taken into account, a genetic influence seems hard to discover from human studies. However, in certain populations and patient groups, the genotype may be the more dominant factor. An important question that so far has received little attention is whether linking of the genotype to magnitude of HVR is of clinical importance. Does a HVR risk phenotype exist which leads to an increased risk for adverse respiratory events? And is this phenotype linked to an increase in morbidity and mortality? This is, for example, of importance for patients with central or obstructive sleep apnea or patients receiving respiratory depressants in the perioperative setting.

Finally, the method of measuring the HVR, especially in humans, remains a topic of intense discussion. We propose a standardized method (the Leiden protocol) that allows measurement of the HVR with as few problems as possible in its interpretation. Adoption of this protocol will enable simple comparisons of physiological and pharmacological studies on HVR between laboratories.

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