HIGHLIGHTED TOPIC | Oxygen Sensing in Health and Disease

Hyperoxia, reactive oxygen species, and hyperventilation: oxygen sensitivity of brain stem neurons


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Oxygen is required to sustain life, but too much oxygen is toxic to the mammalian central nervous system (CNS) due to excessive production and accumulation of reactive oxygen species (ROS). Recall that “oxygen pressure in the mammalian CNS is maintained at a level which is sufficiently high to ensure undisturbed function of brain cells and sufficiently low to minimize generation of free radicals” (24). Regardless, high-dose O2 is routinely, but prudently, used in clinical and nonclinical settings to treat or prevent hypoxemia and tissue hypoxia, and controversially for other miscellaneous disorders (3, 9, 33, 64). Clinical symptoms of CNS O2 toxicity are manifested only when exposure to hyperoxia at normobaric pressure (pure O2 at ~1 ATA) also has subtle but damaging effects on neurological function in the immature CNS (2, 30, 31) and a modulating influence on neuronal excitability in the mature CNS (6, 7, 60).

One neural system that is particularly sensitive to an increase in inspired O2 is the respiratory control system. The ventilatory response to hyperoxia is a biphasic response. The first few breaths of hyperoxia typically decrease expired minute ventilation (Ve) by inhibiting the peripheral chemoreceptors (21, 34, 68, 69). This initial effect of hyperoxia on breathing, however, is short-lived, and continued breathing of the hyperoxic gas mixture for >1–2 min reveals a secondary increase in Ve. The secondary response is called “hyperoxic hyperventilation,” and it occurs primarily through a dose-dependent increase in tidal volume (VT), which may or may not be accompanied by an increase in respiratory frequency (frespir) (4, 14, 35, 36). In some animal studies, the secondary increase in Ve is not observed unless the peripheral chemoreceptors are denervated (27, 41). Because hyperoxic hyperventilation is present in animals with peripheral chemoreceptor denervation (14, 27, 41, 69), the hyperventilation most likely is of central origin. In contrast, the...
initial hypoventilation or apnea is abolished by peripheral chemoreceptor denervation, which confirms that the early transient response is completely dependent on peripheral chemoreception (69).

Hyperoxic hyperventilation is a paradox in respiratory control. Unlike the hypoxic ventilatory response, which functions to maintain tissue oxygenation during hypoxemia (28, 29, 68), the role of hyperoxic hyperventilation in O2 homeostasis is unclear. The maximum level of inspired Po2 (Pio2) in our natural environment is only 160 Torr (0.21 O2 × 760 Torr at sea level = 160 Torr O2 = 0.21 ATA O2). Regardless, understanding the central effect of hyperoxia on respiratory-related neurons (21, 44, 57, 72) becomes very important considering the pervasive use of hyperoxia in respiratory control research (4, 13, 16, 27, 71), clinical medicine (3, 9, 57, 64), and civilian and military professions (18).

This paradox of ventilatory control, we believe, is significant in that it provides clues into the fundamentally important role of reduction-oxidation (redox) reactions, oxidative enzymes, and highly reactive end products, which include ROS (3, 22), in control of breathing (see, e.g., Refs. 37, 38, 40). A similar role for redox signaling and ROS has been identified in O2 sensing by peripheral chemoreceptors (50). In addition, redox signaling and ROS are a general mechanism employed by many cell types to optimize matching between O2 demand and O2 supply in various organs (1). The fact that hyperoxic hyperventilation occurs at all suggests that brain stem neurons regulating breathing are sensitive to oxidative environments (44). Many neurons in respiratory control nuclei of the brain stem contain oxidative enzymes and O2-derived second messengers that are thought to participate in normal cell-signaling pathways (10, 42, 56, 74). It is conceivable that respiratory-related neurons capable of redox signaling are stimulated and/or inhibited by hyperoxia (Pio2 > 160 Torr or > 0.21 ATA O2), with the net effect being stimulation of V E (i.e., hyperoxic hyperventilation). Mulkey et al. (44) recently proposed a similar hypothesis, explaining, in part, hyperoxic hyperventilation. These authors reported that hyperoxia, ROS, and cellular oxidation directly stimulated firing rate of putative central CO2 chemoreceptors in the solitary complex (SC) in rat brainstem slices. In this context, recall that hyperoxia is a commonly used experimental protocol in studies of oxidative stress at normobaric pressure (3, 6) and hyperbaric pressure (19, 20, 49, 65).

A goal of this mini-review, therefore, is to summarize the effects of hyperoxia on V E given its common usage, as cited above. A second goal is to summarize the cellular mechanisms that are proposed to explain the paradox of hyperoxic hyperventilation. In particular, we will focus on recent evidence supporting the hypothesis that central CO2 chemoreceptors are stimulated directly by ROS during hyperoxia (44). A final goal is to examine the practical implications of brain stem O2 and ROS sensitivity relative to our present understanding of common hyperoxic exposures and to suggest future research directions. The importance of the brain stem’s sensitivity to redox signaling and oxidative stress, respectively, in normal and abnormal functioning of the respiratory control network remains to be determined, but accumulating evidence suggests that it is probably very important (13, 32, 37–39, 44, 46, 51, 53, 54, 70, 71). From this perspective, it is appropriate to recall the following passage (67): “Interest in studies of oxygen toxicity is not limited to those who contemplate the exotic environments of sea and space. Such studies may lead to clarification of oxygen effects in normal man at sea level and in patients with cardio-respiratory and other diseases.”

NORMOXIA VS. HYPOXIA AND ROS PRODUCTION

Hyperoxia is defined as any level of Po2, inspired (in vivo) or superfusate (in vitro), that produces a neural tissue Po2 (PtiO2) greater than the range of PtiO2 values that occur in an animal breathing normobaric air (Pio2 of ~160 Torr) (18, 43, 44). Depending on the region of the CNS, normoxic values for PtiO2 will range from a minimum of ~3 Torr up to a maximum of ~35 Torr (18, 43). Therefore, we will consider any value of PtiO2 >35 Torr (Pio2 of >160 Torr) as hyperoxic and capable of increasing ROS levels in the brain stem (6, 19, 20, 25, 65). The O2 continuum that humans routinely encounter in medical and professional settings is very broad, extending up to at least Pio2 = 2,280 Torr (equal to 3 ATA pure O2), which is called “hyperbaric oxygen” (HBO2) (18, 64). At ≤3 ATA O2, the direct effect of pressure per se on the brain stem is thought to be minimal (18, 45). Consequently, any effects of HBO2 on the brain stem are attributed to increased PtiO2 and ROS and not to pressure per se (17, 18, 44, 45).

During hyperoxia, as PtiO2 increases (18, 43), the body’s antioxidant defenses are overwhelmed by increased production of ROS in the mitochondria, nucleus, cytosol, membranes, and extracellular fluid compartments (3, 12). ROS include various O2 free radicals and their reactive by-products, such as superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide, and peroxynitrite (19, 20, 23, 48, 49, 65); this list is by no means complete (3, 22). As reviewed elsewhere (3, 18, 22), ROS can react with various cellular targets, including lipid bilayers (lipid peroxidation), ion channels, and various enzymatic systems, resulting in changes in membrane excitability, synaptic transmission, gene induction, and cellular metabolism. How acute and chronic exposures to excess molecular O2 and ROS affect specific populations of brain stem neurons in the respiratory centers remains to be determined. Initial studies, however, indicate that not all neurons in respiratory-related areas of the brain stem are equally sensitive to hyperoxia and ROS (18, 44, 46). Moreover, previous research, which is summarized next, indicates that a broad range of hyperoxia and ROS stimulates ventilation, suggesting that the respiratory control system responds to a continuum of O2 (and ROS) that ranges from anoxia/hypoxia up through normoxia and hyperoxia.

EFFECTS OF HYPEROXIA ON RESPIRATION

Historically, the direct actions of hyperoxia and ROS on the brain stem at normobaric pressure have rarely been considered when interpreting respiratory control data collected under hypoxic conditions. The popular interpretation when using hyperoxia under in vivo conditions is that any change in V E is related to “chemical denervation” or “physiological chemodenervation” of the peripheral chemoreceptors (see, e.g., Refs. 4, 8, 11, 28, 29, 71). Likewise, for in vitro studies (e.g., brain slices), the popular assumption is that the hyperoxic superfusates (25, 43), which are used routinely as the control O2 tension, have no effect on baseline activity or neuronal responsiveness to the stimulus being tested (see Ref. 18; however, see Refs. 25 and 60 for exceptions to this assumption). Conversely, when subjects are breathing HBO2, the cumulative effects of
ROS are well recognized because of their role in CNS O2 toxicity (3, 19, 20, 64–66). Accumulating evidence supports the notion that the CNS responds to a continuum of O2 tension at both normobaric and hyperbaric pressures (6, 29, 34, 60, 73). If true, then we anticipate that the deleterious effects of HBO2 on the brain stem (14, 61, 66) are preceded by early neuronal events beginning at lower levels of PtcO2. We propose that hyperoxic hyperventilation is an example of the brain stem’s high sensitivity to ROS, possibly an early physiological response in what eventually develops into impaired respiratory distress and failure during CNS O2 toxicity at large doses of HBO2 (14, 44, 61, 66). It follows then that the direct effects of O2 on the brain stem need to be considered when hyperoxia is used. Dripps and Comroe (21) first introduced the use of normobaric hyperoxia (100% O2, 8-min exposure) as a tool for physiological denervation of the carotid body chemoreceptors. They emphasized, however, the following caveat: “It must be remembered that a stimulant effect of oxygen which tends to increase respiration may be acting simultaneously to limit the extent of this immediate depression of minute ventilation.”

The “stimulant effect of oxygen which tends to increase respiration” (21) is well documented in human infants (16, 56, 58) and adults (4, 15, 21, 28, 35, 36, 55, 57) and various animal models (27, 41, 69). Because the dose of hyperoxia, and therefore ROS, is defined by the duration of the hyperoxic exposure and the level of hyperoxia, it is useful to examine studies that have used short and long exposures as well as different levels of hyperoxia at normobaric and hyperbaric pressures. At normobaric pressure, onset of O2 breathing usually evokes an initial transient hypoventilation (21, 69), which is not always observed (4, 27, 41, 55, 57). Within 1–2 min, Ve begins to increase and is significantly elevated above the preexposure level within 5 min (28). Short exposures to isocapnic hyperoxia for only 5 min increase Ve, on average, by ~16% (28). By comparison, 5 min of isocapnic hyperoxia (8–10% O2) increases Ve, on average, by ~37% (28).

Longer exposures to isocapnic hyperoxia reveal a larger and sustained hyperventilation that is dose dependent (4, 55, 57). Figure 1 shows the magnitude of the ventilatory response to graded levels of isocapnic hyperoxia breathed for 30 min [solid lines; values given in parentheses show the relative lack of change in end-tidal Pco2 (PeTCO2) in Torr during isocapnic hyperoxia]. Switching from 21% to 30, 50 and 75% O2 increased Ve, respectively, by 21, 61, and 115% (4). Figure 1 also shows that the magnitude of the hyperoxic hyperventilation is greatly reduced if PeTCO2 is not regulated (i.e., poikilocapnic hyperoxia) (4). In this example, breathing 30 min of 75% poikilocapnic hyperoxia decreased PeTCO2 from 41.3 Torr (air) to 37.7 Torr and increased Ve by only 16% (dotted line in Fig. 1). In this same example (Fig. 1), Ve increased by 115% during 75% isocapnic hyperoxia. Similar results were reported with 55 min and 8 h of isocapnic hyperoxia vs. poikilocapnic hyperoxia (55, 57) [for 8 h of hyperoxia, end-tidal Pco2 was maintained at 300 Torr so as to avert pulmonary O2 toxicity (55)]. The present interpretation is that O2-induced hyperventilation results in hypocapnia, which combines with reduced activity by the peripheral chemoreceptors in high O2 to blunt the magnitude of the O2-induced hyperventilatory response (4, 57). In some cases, hyperventilation is completely masked during poikilocapnic hyperoxia compared with isocapnic hyperoxia (55). This may explain why the hyperoxic hyperventilation response has been missed by many investigators who have used poikilocapnic hyperoxia in their research.

In addition, the example in Fig. 1 illustrates that the ventilatory response to 30 min of isocapnic hyperoxia, at all levels of PtcO2 tested, is not immediately reversible, suggesting that O2 evokes a long-lasting facilitation of Ve. In this context, it is interesting to note that hyperoxia has another time-dependent stimulatory effect on respiration, which is dependent on the activity of neuronal nitric oxide synthase: hyperoxic potentiation of the hypoxic ventilatory response (29). Prebreathing 760 Torr O2 (1 ATA O2) for only 10 min increases the magnitude of the subsequent ventilatory response to isocapnic hypoxia, indicating that “hyperoxia may alter tonic outputs for central respiratory drives” (29).

Under hyperbaric conditions, hyperoxia has similar stimulatory effects on ventilation, at least initially. Prolonged exposure to high levels of HBO2 evokes irregular breathing and respiratory distress and, ultimately, respiratory failure in a dose-dependent fashion (61, 66). In 1921, Dautrebande and Haldane (15) reported that, for subjects who breathed 760 and 1,580 Torr (1.0 and 2.08 ATA) O2 for 5 min, PeTCO2 was decreased by 1.5 and 3.5 Torr, respectively, which they attributed to the central effect of O2 causing graded hyperventilation (72). Lamberts et al. (36) showed that breathing 2,660 Torr (3.5 ATA) poikilocapnic hyperoxia increased Ve by 26%, primarily by increasing Vt, which then decreased PeTCO2 by 6.7 Torr. In anesthetized rats, 30–60 min of 3,040, 4,560, 6,080 Torr (4.0, 6.0 and 8.0 ATA) O2 produced a dose-dependent increase in Ve by increasing both Vt and fresp. Sectioning the glossopharyngeal nerves abolished the fresp response, but not the Vt response, to HBO2. Overall, a significant hyperventilation was still observed during O2 breathing at 4.0 and 6.0 ATA O2 but not at 8.0 ATA O2 because of a large reduction in fresp. Presumably, the inhibitory effects of 8.0 ATA O2 on fresp were due to CNS O2 toxicity (3, 61). Together, these findings and others (27, 41, 44) strongly suggest that hyperoxic hyperventilation is of central origin.
Indeed, prolonged exposure to HBO₂ has deleterious effects on respiration. Simon and Torbati (61) found that continuous exposure to 2,280 and 3,800 Torr (3 and 5 ATA) O₂ has profound effects on f_{resp} (Vt was not measured) in awake rats. Rats who breathed 2,280 Torr (3 ATA) O₂ had increased f_{resp} above air control levels for ~2 h, after which f_{resp} declined to a minimum level (below air control) by 3 h. Next, f_{resp} increased, in some cases, above the air control level, and clinical convulsions occurred along with shallow breathing, gasping, and restlessness. This period of respiratory distress was followed by respiratory failure and usually death after 6 h. Breathing 3,800 Torr (5 ATA) O₂ induced the initial hyperventilation, but f_{resp} became erratic and rapidly decreased after ~15–20 min of HBO₂ breathing, coincident with seizures and followed thereafter by unstable breathing. In the context of disordered breathing patterns caused by HBO₂, it may be significant that normobaric hyperoxia, under certain conditions (e.g., sleep, periodic breathing), also destabilizes breathing, causing irregular breathing patterns and apnea (5, 13, 71).

In summary, these studies reveal several important features of O₂ sensitivity of the respiratory control system as revealed by the ventilatory response to hyperoxia. 1) The brain stem areas controlling ventilation are extremely sensitive to increased P₈O₂. Increasing O₂ from 21% to only 30% while maintaining P_{ET}CO₂ isocapnic caused a significant increase in Vₜ. The increase in Vₜ typically occurs within 1–2 min of exposure to hyperoxia. 2) Increasing P₈O₂ further from 0.21 to 0.50, 0.75, and 1.0–8.0 ATA O₂ shows that the initial hyperventilatory response to O₂ is dose dependent. 3) The ventilatory response to 30 min of isocapnic hyperoxia is not immediately reversible, suggesting O₂ evokes a long-lasting facilitation of breathing (4, 29). 4) The hyperventilatory response shows only slight adaptation after 8 h when care is taken to avert pulmonary O₂ toxicity at normobaric pressure (57). Conversely, prolonged exposures to HBO₂ initially stimulates breathing but then leads to respiratory distress and, ultimately, respiratory failure. 5) O₂-induced hyperventilation significantly reduces P_{ET}CO₂ (4, 16, 35, 36, 57), which blunts or masks (55) the magnitude of the O₂-induced hyperventilation. Thus hyperoxic hyperventilation is best measured during isocapnic hyperoxia (4, 55, 57).

PREVIOUSLY PROPOSED MECHANISMS OF HYPEROXIC HYPERVENTILATION

Seven potential mechanisms have been proposed over the years to explain hyperoxic hyperventilation. The most frequently cited hypotheses are that hyperoxia 1) decreases cerebral blood flow (CBF) and 2) decreases formation of reduced hemoglobin, thereby interfering with CO₂ transport (the “Hal dane effect”). The net effect is to increase PCO₂ in the cerebral vasculature and neural tissue, which stimulates central CO₂ chemoreceptors (16, 35, 36, 55). 3) Lactic acidosis resulting from HBO₂-induced histoxic hypoxia within the brain stem may also stimulate central chemoreceptors (14). These effects of CO₂ and H⁺ are likely to be counterbalanced by the rapidly ensuing respiratory alkalosis that results from O₂-induced hyperventilation (16). 4) In addition, it was proposed that O₂ disinhibits an inhibitory input that is normally active in normoxia (41). 5) Long exposures to hyperoxia, resulting in pulmonary O₂ toxicity, result in pulmonary congestion, irritation, and atelectasis, which may stimulate respiratory reflexes, leading to hyperventilation (cited in Refs. 16 and 35). Pulmonary O₂ toxicity will be a factor with long exposures to hyperoxia, but it is not expected to contribute to the rapid hyperventilation that occurs after only 1–2 min of O₂ breathing (28, 6) It has also been proposed that O₂ directly stimulates respiratory neurons, which results in increased ventilation. No data were provided to support this hypothesis or which type of neuron may be involved (14, 41).

Recently, Mulkey et al. (44) provided the first evidence that neurons in the SC of the dorsocaudal medulla oblongata are directly stimulated by hyperoxia. The following section summarizes the primary findings in their study, which forms the basis for the hypothesis that 7) hyperoxic hyperventilation is initiated by increased production of ROS during hyperoxia, which directly stimulates central CO₂ chemoreceptors in the brain stem (44).

O₂ AND ROS DIRECTLY STIMULATE PUTATIVE CO₂ CENTRAL CHEMORECEPTORS

The SC is one of several sites of CO₂ chemoreception distributed throughout the brain stem (47, 59). Neurons in chemosensitive areas, such as the SC, share several features that suggest a common function in CO₂ chemoreception (59). One of these features is the overwhelming tendency of certain neurons in chemosensitive areas to exhibit an increased firing rate and increased membrane input resistance (i.e., net decreased membrane conductance) during hypercapnic acidosis that is strongly dependent on intracellular pH (pHi) (26, 59).

In the study by Mulkey et al. (44), HBO₂ was used to examine the effects of hyperoxia on SC neurons in the brain slice preparation. Normobaric hyperoxia was not used because the control level of O₂ used in all brain slice studies is, by definition, already hyperoxic (0.95 O₂ × 1 ATA = 0.95 ATA O₂) (18, 25, 43). Consequently, HBO₂, exposing brain slices to O₂ at barometric pressure >1 ATA (17, 43, 44), is the only way to study the effects of further hyperoxia on neurons in the brain slice model, at least until a newer, lower (more physiological?) level of control O₂ is established experimentally for the brain slice preparation (18, 25). The levels of HBO₂ used (2–3 ATA O₂) to test for O₂ sensitivity produced a range of P_{TIC₈} in the slice equivalent to the level of P₈O₂ in the intact brain of a rat exhibiting signs of CNS oxygen toxicity (18, 43, 44). Thus the test levels of O₂ used in these studies (17, 18, 43, 44) were physiologically relevant for evaluating O₂ sensitivity of neurons.

The three main findings in the study of Mulkey et al. (44) were as follows. 1) Not all neurons in the SC are equally sensitive to hyperoxia. Sixty-two percent of the neurons tested showed no significant change in their excitability during ~10 min of hyperoxia ranging from 2 to 3 ATA. Thirty-eight percent of the SC neurons tested, however, were stimulated by hyperoxia, showing increased membrane input resistance and increased firing rate. 2) The neurons stimulated by hyperoxia were the putative central CO₂ chemoreceptors: 90% of the O₂-sensitive neurons were also stimulated by hypercapnic acidosis, whereas 81% of the O₂-insensitive neurons were CO₂ insensitive. Figure 2A shows the integrated firing rate response of a SC neuron that was stimulated by normobaric hypercapnic acidosis (left) and hyperbaric hyperoxia (right) [the cell...
showed no significant response to pressure per se on compression with the inert gas, helium; not shown (45)]. When CO₂-excited neurons were exposed to hyperoxia, there was usually a small increase in membrane input resistance, a small depolarization, and a significant increase in firing rate (44). 3) The ability of hyperoxia to increase firing rate and input resistance could be blocked by coexposure with an antioxidant and mimicked by exposure to chemical oxidants at control levels of P O₂. Figure 2B shows the firing rate response of an O₂-sensitive neuron that was blocked, reversibly, by coexposure with Trolox-C (a membrane-permeable analog of vitamin E). The present working model is that hyperoxia increases ROS, which inhibit a potassium channel(s), although the specific ionic targets have not been identified (17, 44, 46).

The above results indicate that putative central CO₂ chemoreceptors will be the first neurons in the SC affected by breathing hyperoxia, suggesting that they are especially sensitive to O₂ and ROS. This may explain why, in humans, there is a significant linear correlation (r = 0.83) between the magnitude of the ventilatory response to hypercapnia and the magnitude of the ventilatory response to 0.75 ATA isocapnic hyperoxia (4). The above findings also are noteworthy in that they provide the first evidence that stimulation of putative central CO₂ chemoreceptors occurs in the absence of any secondary changes in P CO₂ caused by decreased CBF and diminished reduction of hemoglobin (Haldane effect), which, until now, have been the favored mechanisms for explaining hyperoxic hyperventilation (4, 16, 35, 36, 56, 57).

In the intact animal, however, the cumulative effects of O₂ and ROS and CO₂/H⁺ (due to decreased CBF, Haldane effect, and so forth) on the brain stem are additive, especially during poikilocapnic hyperoxia. Figure 3 shows that the combined effect of CO₂ and O₂ on putative central CO₂ chemoreceptors is to produce a large stimulation of firing rate (44). In these experiments, each neuron was stimulated by exposure to hypercapnia alone (Fig. 3, A–C), hyperoxia alone (Fig. 3, A and B), or chemical oxidant alone [Fig. 3C; N-chlorosuccinimide (NCS)]. When the same level of hypercapnia was combined with hyperoxia (Fig. 3, A and B) or NCS (Fig. 3C), the firing rate response of each cell was at least additive (Fig. 3B) and often greater (Fig. 3, A and C). Recent research indicates that chemical oxidants also have a large effect on pH i of SC neurons, causing a significant intracellular acidification and slowing of Na⁺/H⁺ exchange (46). The bases for these interactions between O₂, ROS, CO₂, and pH i require further investigation.

**FUTURE DIRECTIONS FOR RESEARCH**

There are at least three implications of brain stem O₂ sensitivity that need to be considered in future studies given the present uses of hyperoxia in clinical and basic research. First, experiments that use O₂ breathing have to take into account the central effects of increased O₂ (4, 21). Future research should
establish the temporal relationship between electrical signaling in neurons in central chemosensitive areas and electrical signaling by peripheral chemoreceptors during hypoxia and hypoxia. Until that occurs, it has to be assumed that the initial hypventilation [peripheral chemoreceptor inhibition by O₂ and ROS (34, 68)] may be limited by the simultaneously occurring stimulation of Ve [central chemoreceptor stimulation by O₂ (44) and the secondary increase in CO₂/H⁺ (4, 16, 35, 36)], as first proposed by Dripps and Comroe in 1947 (21). Thus caution should be exercised in attributing any change in Ve during hypoxia solely to “peripheral chemo-denervation.” For example, the central effects of O₂ and ROS may be a factor in studies of periodic breathing (71), assessing peripheral chemoreceptor function in infants (8), characterizing the hypoxic ventilatory response (29), and studying interactions between the peripheral CO₂ and central CO₂ chemoreceptors (11). In particular, if O₂ breathing lasts for more than 1–2 min, the increase in Ve will affect results of ventilatory response tests (4). In addition, the ensuing arterial hypoxemia from hypoxic hyperventilation will alter cerebral vascular tone and thus CBF and intracranial pressure. This latter issue becomes especially important when considering the effects of O₂ therapy on CBF and intracranial pressure in cases of brain trauma (57). As previously stated (4, 57), it is premature to consider altering the present O₂ therapy protocols to account for the effects of hypoxic hyperventilation on CBF and acid-base balance until the appropriate clinical trials are conducted.

Second, because hypoxia is a useful model of oxidative stress (3, 6, 18) and perturbs breathing, as discussed above, future research will want to determine how O₂ and other oxidative conditions affect respiratory control. If redox signaling is key to normal respiratory control, then excessive amounts of oxidative stress may negatively impact normal cellular mechanisms of control. Thus it will be important in future studies to determine the effects of acute and chronic oxidative stress on respiratory rhythmosgenesis and the various afferents that alter ventilation, for example, peripheral and central chemoreceptors (34, 42, 44). In addition, future studies will want to determine the effects of a broad range of O₂ on neural activity and the mechanisms of hypoxia-induced changes in neuronal plasticity, such as long-term facilitation (4, 29). Furthermore, it also will be important to determine the effects of oxidative stress over the course of development and maturation of the respiratory control system. In these contexts, it may be significant that normobaric hypoxia increases the incidence of sleep-related apneic episodes in rats (13), introduces periodic breathing and destabilizes breathing further during periodic breathing in lambs (71), and destabilizes breathing in infants presenting with recurrent apnea and cyanosis (5). Likewise, other forms of chronic oxidative stress, caused by metabolic defects, may render the developing brain stem network vulnerable to impaired functioning under certain conditions (32, 54, 62). For example, high levels of iron (53, 70) and low levels of melatonin (39) result in chronic oxidative stress and occur in children that die from sudden infant death syndrome.

Finally, because of extensive use of in vitro tissue preparations of the CNS in respiratory control studies, and neuroscience in general, it is appropriate to consider the potential effects of a hyperoxic control solution on neuronal function. As discussed elsewhere (18), nearly all brain slice experiments are conducted under conditions of hypoxia with 95% (0.95 ATA) O₂ (43). The PtiO₂ profile in a submerged, 300-μm-thick rat brain stem slice is equivalent to the PtiO₂ in the cortex of a rat breathing 2.2–2.9 ATA O₂ (18, 43). This means that the control PtiO₂ level used in nearly all brain slice studies is effectively “HBO₂” minus the physical pressure component (18). What effect this level of hypoxia has on ROS production in various in vitro preparations requires further study (18, 25). However, the fact that hypoxia has long-lasting effects on ventilation (4, 29), and selectively stimulates certain populations of SC neurons (44), is certainly a cause for concern. For example, recent pHᵢ measurements in brain stem slices indicate that pHᵢ of neurons in the SC, specifically within the nucleus tractus solitarius, decreases as PtiO₂ decreases (mean ± SE): 95% O₂ (7.44 ± 0.01), 60% O₂ (−0.10 ± 0.01 ΔpH unit, where Δ indicates change), 20% O₂ (−0.16 ± 0.01 ΔpH unit), and 0% (−0.16 ± 0.01 ΔpH unit) (Potter SJ, Putnam RW, and Dean JB, unpublished findings). This demonstrates that the use of a lower level of control O₂ will affect “control” pHᵢ in SC neurons, which is anticipated to significantly affect excitability of CO₂-chemosensitive neurons and any other pH-dependent processes that occur in other SC neurons.

Furthermore, levels of O₂ in in vitro preparations of the CNS that are often referred to as hypoxia are in fact physiological (PtiO₂ < 35 Torr) (18, 43), and accounting for this fact may alter our interpretation of the responses of the brain to true hypoxia. Given the significant effects that elevated O₂ levels have on ventilation, further studies of the role of hypoxia on brain stem neurons are clearly of interest. Identifying the effects of the O₂ continuum on basic cellular properties, which includes anoxia/hypoxia through normoxia and hypoxia, we will gain insight into the O₂ sensitivity of the brain stem and the role that redox signaling plays in respiratory control (1, 22, 37, 38, 44) as well as the possible role for oxidative stress in diseases of respiratory control (22, 32, 39, 53, 54, 62, 67, 70).

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Invited Review

O₂ SENSING: THE HYPOXIC HYPERVENTILATION PARADOX


