HIGHLIGHTED TOPIC | *Central CO₂ Chemoreception in Cardiorespiratory Control*

Hypercapnia causes cellular oxidation and nitrosation in addition to acidosis: implications for CO₂ chemoreceptor function and dysfunction

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Cellular mechanisms of CO₂ chemoreception are discussed and debated in terms of the stimuli produced during hypercapnic acidosis and their molecular targets: protons generated by the hydration of CO₂ and dissociation of carbonic acid, which target membrane-bound proteins and lipids in brain stem neurons. The CO₂ hydration reaction, however, is not the only reaction that CO₂ undergoes that generates molecules capable of modifying proteins and lipids. Molecular CO₂ also reacts with peroxynitrite (ONOO⁻), a reactive nitrogen species (RNS), which is produced from nitric oxide (NO) and superoxide (O₂⁻). The CO₂/ONOO⁻ reaction, in turn, produces additional nitrosative and oxidative reactive intermediates. Furthermore, protons facilitate additional redox reactions that generate other reactive oxygen species (ROS). ROS/RNS generated by these redox reactions may act as additional stimuli of CO₂ chemoreceptors since neurons in chemosensitive areas produce both NO and O₂⁻ and, therefore, ONOO⁻. Perturbing NO, O₂⁻, and ONOO⁻ activities in chemosensitive areas modulates cardiorespiration. Moreover, neurons in at least one chemosensitive area, the solitary complex, are stimulated by cellular oxidation. Together, these data raise the following two questions: 1) do pH and ROS/RNS work in tandem to stimulate CO₂ chemoreceptors during hypercapnic acidosis; and 2) does nitrosative stress and oxidative stress contribute to CO₂ chemoreceptor dysfunction? To begin considering these two issues and their implications for central chemoreception, this minireview has the following three goals: 1) summarize the nitrosative and oxidative reactions that occur during hypercapnic acidosis and isocapnic acidosis; 2) review the evidence that redox signaling occurs in chemosensitive areas; and 3) review the evidence that neurons in the solitary complex are stimulated by cellular oxidation.

reactive oxygen species; reactive nitrogen species; carbon dioxide; central chemoreception; hypercapnia

MOLECULAR O₂ AND MOLECULAR CO₂ GENERATE REACTIVE OXYGEN SPECIES/REACTIVE NITROGEN SPECIES

MOLECULAR OXYGEN is a strong oxidant that is reduced sequentially to yield oxygen free radicals and their highly reactive nonradical derivatives. Known as “reactive oxygen species” (ROS), these strong oxidants include superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH) among others (22). Molecular oxygen also reacts with l-arginine in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and nitric oxide synthase (NOS) to yield the free radical nitric oxide (NO) and citrulline (45, 52). Nitric oxide is an important signaling molecule in the central nervous system (CNS) and a “reactive nitrogen species” (RNS). Nitric oxide reacts with O₂⁻ to produce peroxynitrite (ONOO⁻), which is yet another type of RNS. These various oxidation-reduction (i.e., redox) reactions occur under normal physiological conditions and contribute to normal mechanisms of cellular signaling (22, 26). Under adverse conditions, redox reactions can also produce excessive levels of ROS/RNS that exceed the body’s endogenous antioxidant defenses resulting in “oxidative and nitrosative stress” and cellular dysfunction (22, 80). Not surprisingly, ROS and RNS production have been implicated in the brain stem’s sensitivity to hypoxia (13, 36, 40, 47, 62) and hyperoxia (16–19, 57).

In this minireview, I propose that molecular CO₂ and H⁺ are two additional sources of ROS/RNS that modulate brain stem neurons, including those in CO₂-chemosensitive areas. Molecular CO₂ also reacts with ONOO⁻ to generate the RNS called...
neurons, is stimulated by cellular oxidation that causes changes in mechanisms of excitability and pH regulation.

**REDOX AND NITROSATIVE REACTIONS ACTIVATED DURING HYPERCAPNIC ACIDOSIS AND ISOCAPNIC ACIDOSIS**

The reaction between carbon dioxide/bicarbonate and peroxynitrite is important for understanding mechanisms of oxidant-mediated toxicity. First, normal plasma bicarbonate and carbon dioxide concentrations are 25 and 1.3 mM, respectively, but higher levels could be achieved during tissue pathological events such as respiratory distress or ischemia-reperfusion. Second, excess production of peroxynitrite has been documented to occur during these and other instances of accelerated tissue superoxide and nitric oxide production, enhancing the potential for coordinated reaction mechanisms to occur between CO2/HCO3-, peroxynitrite, and tissue target molecules.

—From Denicola et al. (Ref. 21)

**CO2/HCO3- and peroxynitrite.** Peroxynitrite (ONOO-) is formed by the reaction of \( \cdot \)O2 and \( \cdot \)NO (20, 73, 86, 91) [Fig. 1, reaction (Rxn) 3]. The \( \cdot \)O2/\( \cdot \)NO reaction (Fig. 1, Rxn 3) has been called the “radical switch” that diverts \( \cdot \)NO away from its normal regulatory signaling actions toward potentially cytotoxic reactions that result in nitrosative and oxidative damage (27, 86). The \( \cdot \)NO/\( \cdot \)O2 reaction is diffusion limited and occurs more rapidly than the catalyzed dismutation reaction of \( \cdot \)O2 (superoxide dismutase, SOD) to form H2O2 (4) (Fig. 1, Rxn 4). The \( \cdot \)NO/\( \cdot \)O2 reaction that forms ONOO- occurs in both intracellular and extracellular compartments. Peroxynitrite readily crosses the plasma membrane in its protonated form (peroxynitrous acid, ONOOH) to react with intracellular substrates. Peroxynitrite anion also crosses the cell membrane via a 4,4'-disothiocyanato-2,2'-stilbene-disulfonic acid (DIDS)-sensitive anion exchanger that normally functions in Cl-/HCO3- exchange (78); this latter mechanism predominates at physiological pH (89). Once formed, ONOO- undergoes several reactions to yield molecular products of varying reactivity (91): oxidation of sulfhydryl (thiol) groups to disulfides; nitration of aromatic compounds; \( S \)-nitrosylation of sulfur atoms in amino acid residues resulting in posttranslational modification of proteins (i.e., \( S \)-nitrosylated proteins, SNO); promotion to produce peroxynitrous acid that decomposes to form nitrate; and reaction with CO2 to generate nitrosoperoxocarboxylate (ONO2CO2-) (21, 26, 27, 31, 70, 71, 73, 78, 89–91). It is the last reaction between CO2 and ONOO- that occurs ubiquitously in a CO2/HCO3- buffered system (Fig. 1, Rxn 5) and, moreover, that is postulated here to be involved in nitrosative and oxidative modulation of brain stem neurons, including chemosensitive neurons, during hypercapnic acidosis and oxidative stress (Fig. 1, Rxn 6).

Peroxynitrite is unstable in CO2/HCO3- buffered medium and is quickly consumed by its reaction with CO2 to yield ONO2CO2- (21, 70, 71, 89) (Fig. 1, Rxn 5). Addition of the enzyme carbonic anhydrase (CA), which catalyzes the hydration/dehydration reactions of CO2 and HCO3- (Fig. 1, Rxn 7/Rxn 8), also increases the rate of conversion of ONOO- to ONO2CO2- (89) (Fig. 1, Rxn 5). In the presence of CO2/HCO3-, a limited number of molecular targets react directly with ONOO- (27, 70). Instead, biomolecules react with the various RNS intermediates that are produced from the CO2/ONOO- reaction (3, 27, 70, 86, 91). The CO2/ONOO- reaction is one...
of the fastest reactions that ONOO\(^{-}\) undergoes (reaction rate, \(k = 5.8 \times 10^4 \text{ M}^{-1}\cdot\text{s}^{-1} \) at 37°C), and it predominates in the extracellular space where the concentration of sulfhydryl groups is low and CO\(_2\)/HCO\(_3\) concentration is high; that is, formation of ONOO\(_2\)\(\cdot\)CO\(_2\) is favored in the extracellular compartment over formation of disulfides (91). Radi (73) has proposed that over 90% of ONOO\(^{-}\) initially reacts with CO\(_2\) in the extracellular space and from 30–40% of ONOO\(^{-}\) initially reacts with CO\(_2\) in the intracellular space. As stated above, however, ONOO\(^{-}\) has no problem crossing the cell membrane and gaining access to intracellular targets (78).

The CO\(_2\)/ONOO\(^{-}\) reaction occurs over a broad range of pH, including physiologically relevant pH that stimulates breathing in vivo and CO\(_2\)-chemosensitive neurons under in vitro conditions, for example, arterial partial pressure of carbon dioxide (PCO\(_2\)) and pH ranges, respectively, from ~35–45 Torr and ~7.35–7.45. An increase in arterial PCO\(_2\) of only a few Torr significantly increases minute ventilation (34, 59, 81). In rats, switching from air to 6% CO\(_2\) in air decreases cerebrospinal fluid pH (pH\(_{\text{CSF}}\)) from 7.396 to 7.294 and neural pH\(_i\) from 7.044 to 6.982 and stimulates minute ventilation. Breathing 11% CO\(_2\) in air decreases pH\(_{\text{CSF}}\) further to 7.190 and brain pH\(_i\) to 6.910 and stimulates breathing (83). In rat brain stem tissue slices, acute exposures to moderate levels of hypcapnia decreases pH\(_i\) by \(\approx 0.2\) pH units from a baseline pH\(_i\) of ~7.24 (28, 76, 77), decreases membrane conductance, and stimulates firing rate of neurons in CO\(_2\)-chemosensitive areas (14, 28, 42). Based on these studies, the physiologically relevant range of pH\(_{\text{CSF}}\)/pH\(_i\) for stimulation of chemoreceptors encompasses ~7.4 down to 6.91. This range of pH overlaps with the range of pH (6.5–7.4) that the CO\(_2\)/ONOO\(^{-}\) reaction occurs (21, 71, 89).

At pH 7.4, the predominance of the CO\(_2\)/ONOO\(^{-}\) reaction is demonstrated by the decrease in peroxynitrite-mediated oxidation products when CO\(_2\)/HCO\(_3\) was added to the reaction vessel; that is, addition of CO\(_2\)/HCO\(_3\) accelerated consumption of ONOO\(^{-}\) to yield ONOO\(_2\)\(\cdot\)CO\(_2\) before ONOO\(^{-}\) could react with other substrates and oxidize molecular targets such as glutathione and oxyhemoglobin (21). Increasing CO\(_2\) further accelerates consumption of ONOO\(^{-}\) (21, 71, 86, 89, 91).

The CO\(_2\)/ONOO\(^{-}\) reaction redirects the primary reactivity of ONOO\(^{-}\) in both intracellular and extracellular compartments to nitrosative and oxidative reactions through breakdown of ONOO\(_2\)\(\cdot\)CO\(_2\) into carbonate radicals (CO\(_3\)\(\cdot\)) and nitrogen dioxide radicals (\(\cdot\)NO\(_2\)) and other species (70, 71, 91) (Fig. 1, Rxn 6). In the course of producing these derived nitro-oxidants, CO\(_2\) is regenerated and recycled for further production of ONOO\(_2\)\(\cdot\)CO\(_2\) (71) (Fig. 1, Rxn 6). The formation of CO\(_3\)\(\cdot\) and \(\cdot\)NO\(_2\) is favored in the nonpolar environment of the plasma membrane (91). Carbonate radicals are more stable than \(\cdot\)OH and can diffuse from neuron to neuron to activate proximal brain vasculature (71).
reactions are protein oxidations via hydrogen atom abstraction and electron transfer (3). Important targets for CO$_2$• include tyrosine, tryptophan, guanine, cysteine, sulfhydryl groups, and nucleic acids (27, 70, 86, 88, 91). Nitrogen dioxide is a RNS that can react with other radical species or oxidize biomolecules via electron transfer and hydrogen atom abstraction (3). Important targets include proteins, fatty acids, phenols, thiols, tyrosine, tryptophan, and cysteine residues (3, 70).

As stated above, ONOO$^-$ undergoes several other reactions to varying extent in the presence of CO$_2$/HCO$_3^-$ (26, 91). The lack of comment in the literature on the effects of pH on these other reactions suggests that they are either unremarkable in their pH sensitivity or that their pH sensitivity is unstudied to date. Regardless, I mention one of these ONOO$^-$ reactions here—the S-nitrosylation (SNO) reactions—for the following two reasons: 1) S-nitrosylation reactions have been implicated in cardiorespiratory control mechanisms in the nucleus tractus solitarius, which is the dorsal nucleus of the SC (see below) (47, 65); and 2) S-nitrosylation reactions redirect ONOO$^-$ away from reacting with CO$_2$ and therefore influence formation of CO$_2$-dependent RNS (21, 26, 71, 86, 89, 91). Peroxynitrite induces SNO reactions (26, 90) that cause reversible nitrations at cysteine and methionine residues that are thought to be involved in normal signaling mechanisms (31, 87), including cardiorespiratory control (47, 65). Conversely, irreversible SNO reactions occur principally from nitration of tyrosine residues forming nitrosocarbonate and are believed to be involved in pathological processes (31, 35, 88).

H$^+$ and the Fenton reaction. Trace amounts of metal ions, such as iron, are important activators of redox reactions (86). The Fenton reaction (Fig. 1, Rxn 10) describes the iron-catalyzed reaction in which H$_2$O$_2$ is converted to OH$^-$. Ferrous iron (Fe$^{2+}$) is oxidized by H$_2$O$_2$ to ferric iron (Fe$^{3+}$) plus hydroxyl radical and hydroxyl anion: Fe$^{2+}$ + H$_2$O$_2$ $\rightarrow$ Fe$^{3+}$ + OH$^-$ + OH$^-$. Hydroxyl radicals are more reactive and thus damaging than CO$_2$• and NO$_2^-$. They react with essentially all biomolecules that they collide with, which is why the effects of OH$^-$ are more random and widespread compared to the targeted reactions initiated by CO$_2$• and NO$_2^-$. (70). Under normal conditions, oxidative stress from excessive production of OH$^-$ is kept in check, in part, by the high-affinity iron-binding protein, transferrin (Tf), which is present in the CSF, plasma, extracellular fluid, and lymph (30). Binding of iron to Tf is strongly pH dependent (85), and decreasing pH$_i$ increases dissociation of iron from Tf (2, 82) (Fig. 1, Rxn 7b and Rxn 12). At pH$_i$ = 7.4, free Fe$^{3+}$ binds to Tf and the transferrin receptor (TfR1) located on the plasma membrane. The Fe-Tf-TfR1 complex is internalized by endocytosis to form a vesicle (endosome) whose internal pH is maintained acidic (pH ~6.0) via proton pumps. Vesicular acidification causes dissociation of Fe$^{3+}$ from Tf-TfR1, which is reduced to Fe$^{2+}$ by a ferrireductase (63). Ferrous iron then goes to the mitochondrion, or, alternatively, it exits the endosome into the cytosol via the divalent metal transporter, DMT1 (30). Presumably, during acidosis, decreasing pH$_i$ further acidifies vesicular pH causing enhanced release of Fe$^{3+}$ from Tf-TfR1 and reduction to Fe$^{2+}$, which increases the pool of free iron catalyzing the Fenton reaction within the cell (30). Binding of iron to Tf, however, is also dependent on HCO$_3^-$. Transferrin binds ferric ion and one anion, which is usually HCO$_3^-$ (23) (Fig. 1, Rxn 11). Cellular acidosis caused by metabolic acidosis (Fig. 1, Rxn 12), therefore, would favor dissociation of iron from Tf due to concurrent decreases in both pH and HCO$_3^-$ (isocapnic acidosis), thereby providing Fe$^{2+}$ for catalyzing the Fenton reaction and increasing production of OH$^-$. (74, 91).

On the other hand, hypercapnic acidosis would stabilize the Fe-Tf-TfR1 complex in part by producing equimolar amounts of HCO$_3^-$ and H$^+$ (Fig. 1, Rxn 7b), each species having opposing effects on the Fe-Tf-TfR1 complex (74, 91) (Fig. 1, Rxn 13). This is why hypercapnic acidosis is thought to provide neuroprotection during ischemia and hypercapnic hypoxia: increased levels of CO$_2$/HCO$_3^-$ stabilize the Fe-Tf-TfR1 complex and decrease free iron availability for catalyzing redox reactions (74, 91). In addition, as discussed above, rapid conversion of ONOO$^-$ to ONOOH during hypercapnic acidosis effectively decreases the oxidative damage caused by ONOO$^-$ (74, 91). At the same time, however, we recall that the CO$_2$/ONOO$^-$ reaction has its own potentially adverse consequences on cellular function through production of reactive intermediates that cause cellular oxidation and nitrosative processes (3, 5, 27, 33, 70) (Fig. 1, Rxn 5 and Rxn 6).

Acid-activated Fenton chemistry is an important mechanism in modulating neuronal function during pathological conditions such as ischemia, metabolic disorders, and chronically unstable breathing (22, 80, 93). Under more physiological conditions, the importance of the Fenton reaction in CO$_2$ chemoreception during acidosis remains to be determined since, in addition to the iron binding proteins, CO$_2$, and H$_2$O$_2$ disposing systems (catalase, peroxidase) that keep their levels of H$_2$O$_2$ low under normal conditions (22, 38). Having said that, redox signaling can occur in restricted cellular domains that do not reflect the overall redox state of the cell (68). It is conceivable that the Fenton reaction could likewise be restricted to a particular subcellular compartment that enables it to act as a physiological modulator during acidosis of chemosensitive neurons and other cardiorespiratory neurons. For example, possible restricted subcellular domains for targeted redox signaling include the plasma membrane (lipids, proteins, and/or cytoskeleton), cytosol, mitochondrial matrix, or mitochondrial intermembrane space (11, 37, 68). Compartmentalization within a particular nanostructural domain of the cell is thought to be a critical factor in determining if a given redox reaction functions in physiological regulation or as an agent of nitrosative and oxidative stress (68).

Other redox reactions influenced by H$^+$ and CO$_2$/HCO$_3^-$. Changes in pH modulate other redox reactions besides the Fenton reaction (70, 91). For instance, the protonated form of superoxide, H$_2$O$_2$•, which is favored by acidification, has greater reactivity than OH$^-$ and is thought to initiate membrane lipid peroxidation. Protonated superoxide is unchanged and more readily crosses the plasma membrane compared to the charged, impermeable superoxide anion (38). In addition, un consumed H$_2$O$_2$ reacts with SOD, which is the catalytic enzyme that converts OH$^-$ to H$_2$O$_2$ (Fig. 1, Rxn 4). As a result, a reactive intermediate of SOD is produced that behaves as a hydroxyl radical (SOD-Cu$^{2+}$,OH) and, in the process, inactivates SOD (91). In the presence of CO$_2$/HCO$_3^-$, however, H$_2$O$_2$ reacts with the SOD-Cu$^{2+}$,OH to produce active SOD and CO$_2$• (91).

To summarize the first section, in a CO$_2$/HCO$_3^-$-buffered system, increasing the level of CO$_2$ results in formation of carbonic acid and nitrosperoxocarboxylate. Both reactions are
accelerated by carbonic anhydrase and occur in the extracellular and intracellular compartments. Carbonic acid immediately dissociates into $\text{H}^+$ and $\text{HCO}_3^-$ causing cellular acidosis. At the same time, $\text{ONO}_2\text{CO}_2^-$ reacts to generate RNS intermediates that result in cellular oxidation, nitration, and nitrosylation. Likewise, acidosis accelerates other redox reactions, producing additional ROS, particularly during metabolic acidosis. The available pool of stimuli during hypercapnic acidosis and metabolic acidosis, therefore, includes decreased pH and various reactive intermediates that can potentially modulate membrane-bound proteins and lipids (11) through various nitrosative and oxidative reactions. The pool of reactive species produced during respiratory acidosis vs. metabolic acidosis is predicted to be different depending on the respective contributions from Fenton chemistry (proton activated) vs. peroxynitrite chemistry ($\text{CO}_2/\text{HCO}_3^-$ activated). It is tempting to speculate that such differences in redox states could underlie differences in the brain stem chemoreceptor and ventilatory response to respiratory acidosis vs. metabolic acidosis (24).

EVIDENCE OF REDOX AND NITROSATIVE SIGNALING IN BRAIN STEM CHEMOSENSITIVE AREAS

What is the evidence, therefore, that neurons in chemosensitive areas produce the requisite enzymes (NOS, NADPH oxidase, and SOD) and reactants ($\text{O}_2^\cdot$, $\text{H}_2\text{O}_2$, $\text{NO}^\cdot$, and $\text{ONOO}^-$) to drive the $\text{CO}_2/\text{ONOO}^-$ reaction, Fenton reaction, and other pH-dependent redox reactions under physiological and pathological conditions of acidosis? There are multiple areas of CO2-chemosensitivity dispersed throughout the brain stem (59), including the dorsal medulla [solitary complex, SC (15)] and pons [locus ceruleus (LC)], midline medullary raphe, pre-Bötzinger complex (PBC), and ventrolateral medulla (VLM). Of these, only the SC has been investigated to date in the context of how oxidative stress affects putative CO2 chemoreceptors (19, 57, 58). The nucleus tractus solitarius (NTS) is recognized as a site of CO2 chemosensitivity in the dorsocaudal medulla oblongata (59, 81). For reasons discussed elsewhere, I have proposed (15) that the boundaries of the dorsocaudal chemosensitive area in the medulla encompass the caudal NTS and dorsal motor nucleus of the vagus nerve (DMV). Hence, evidence for redox signaling in NTS and DMV, which together comprise the SC, is considered below. While few labs have studied redox signaling as it pertains to CO2 chemoreception, several studies have investigated the mechanisms of ROS/RNS production in chemosensitive areas but from the perspective of understanding redox signaling mechanisms underlying cardiovascular control and dysfunction (8, 32, 41, 61) and the hypoxic ventilatory response (13, 39, 40, 47, 62).

Nitric oxide. Nitric oxide, which is required for ONOO$^-$ production, is produced with L-citrulline from L-arginine in a NOS-catalyzed reaction that uses molecular $\text{O}_2$ and NADPH (45, 52) (Fig. 1, Run 2). NOS immunoreactivity has been localized in the caudal NTS (40, 64, 67), DMV (94), PBC (48), RVL (7, 13, 44, 64, 95), and dorsal raphe (66, 84). Microinjection of $\text{NO}^\cdot$ donor unilaterally into the NTS in awake and unrestrained rats stimulated ventilation in both normoxia and hypoxia, whereas microinjection of NOS inhibitor into the NTS blunted the hypoxic ventilatory response (62). Bilateral injection of NOS inhibitor into the caudal NTS of awake rats increased arterial blood pressure and decreased the ventilatory response to hypoxic activation of the peripheral chemoreceptors (36, 39). In the PBC, 53% of the neurons expressing neurokinin-1 receptor immunoreactivity—the marker for PBC neurons—were colocalized with NOS (48). Other investigations have focused on the larger structure, the RVL. Sympathoexcitatory neurons in RVL also express NOS (44, 95); these neurons are thought to be chemosensitive or to receive input from chemoreceptors in the RTN (53, 54). Nitric oxide has been implicated in RVL control of the hypoxic ventilatory response (13), pressor response (9, 32, 41, 61), and heart failure (8). Serotonergic neurons in the dorsal raphe also express NOS (66, 84).

In addition, S-nitrosothiols are NO donors that produce $\text{NO}^\cdot$, nitrosomium cations ($\text{NO}^+$) and nitroxyl anions ($\text{NO}^-)$ through enzymatic cleavage reactions (31, 90). S-nitrosothiols target neurons in the NTS in a stereoselective fashion for reflex control of blood pressure and the hypoxic ventilatory response. Microinjection of S-nitroso-L-cysteine into the NTS caused a dose-dependent increase in heart rate and arterial blood pressure (65) and mimicked the stimulatory effects of hypoxia on minute ventilation (47). Likewise, microinjection of S-nitroso-cysteinyl glycine into the NTS also mimicked the hypoxic ventilatory response (47).

Superoxide anion. Superoxide anion, which is required for production of ONOO$^-$, is generated from enzymatically catalyzed reactions involving NADPH oxidase and xanthine oxidase (22) (Fig. 1, Run 1). NADPH oxidase was localized in caudal NTS neurons (32, 92), and NADPH oxidase-dependent $\text{O}_2^\cdot$ production was measured in NTS neurons (61). In addition, NADPH oxidase expression increases in RVLM neurons in a stereoselective fashion for reflex control of blood pressure and the hypoxic ventilatory response. NADPH oxidase subunits (p40phox, p47phox and gp91phox) are expressed in RVL neurons and their expression increases during chronic heart failure. Tempol, a $\text{O}_2^\cdot$ scavenger, inhibits development of experimental chronic heart failure and decreases blood pressure in control and hypertensive rats (29). Peroxynitrite. The only chemosensitive region in which ONOO$^-$ activity has been measured is the RVL; no other chemosensitive area has been investigated to my knowledge. In the RVL, ONOO$^-$ has been implicated in fatal cardiovascular depression (7, 8) and endotoxin-induced apoptosis (25). That said, it is plausible that ONOO$^-$ is synthesized in other chemosensitive areas given reports that NOS/NO and NADPH oxidase/$\text{O}_2^\cdot$ and SOD are localized in a number of chemosensitive areas (see above). For example, formation of...
ONOO⁻ is thought to be regulated by SOD, which can lower the concentration of •O₂⁻ (Fig. 1, Rxn 4) available for producing ONOO⁻ (86). Superoxide dismutase has also been identified in the SC (61, 94). Likewise, •NO and •O₂⁻ activity have been identified in SC (16, 17, 32, 61, 92).

Fenton reaction. Cellular acidosis accelerates the Fenton reaction (Fig. 1, Rxn 10) by increasing dissociation of iron from Tf (Fig. 1, Rxn 11), making it available for reducing H₂O₂ to •OH. Transferrin is located throughout the medulla and pons (1, 55), with high concentrations noted in the LC (55), dorsal raphe (56), and SC (49, 55). Hydrogen peroxide, which reacts with iron in the Fenton reaction, is produced from •O₂⁻ via the dismutase reaction catalyzed by SOD (22) (Fig. 1, Rxn 4). The presence of SOD, therefore, indicates ongoing production of H₂O₂ from •O₂⁻ (and also possible control of ONOO⁻ production, as discussed above (86)). To date, •O₂⁻ production has been demonstrated in the SC (16, 17, 32, 61, 92) and RVLM (9, 32, 60). Immunoreactivity for manganese-superoxide dismutase (Mn-SOD) and copper/zinc-superoxide dismutase (Cu/Zn-SOD) was detected in SC neurons (61, 94). Transfection of adenovirus vectors encoding the Mn-SOD gene or Cu/Zn-SOD into the NTS reduced blood pressure in spontaneously hypertensive rats (41). Injection of H₂O₂ into the NTS induced a dose-dependent, transient bradycardia and hypotension in anesthetized rats. Likewise, injection of catalase inhibitor, which delays conversion of H₂O₂ to H₂O, thereby allowing accumulation of endogenously produced H₂O₂, caused a similar bradycardia and hypotension (6). The ANG II-induced pressor response elicited from RVLM neurons involves Ca²⁺-dependent increase in mitochondrial •O₂⁻ production. Overexpression of Mn-SOD, which accelerates conversion of •O₂⁻ to •OH, decreases the magnitude of ANG II-induced ROS production and the pressor response (60).

To summarize the second section, the data, which come mostly from studies of the SC and RVLM, indicate that neurons in chemosensitive areas express NOS, NADPH oxidase, and SOD under baseline conditions and employ signaling mechanisms that use •O₂⁻, •NO, ONOO⁻, and SNO. Perturbation of these redox signaling mechanisms through their activation or inhibition produces alterations in cardiorespiratory output. The relative contribution of these redox reactions to CO₂ chemoreception, however, is untested to date. It will be important to determine how perturbation of these redox reactions affects neuronal chemosensitivity in vitro as well as the ventilatory response to hypercapnia in intact and chemodenervated animals. For example, one could study mechanisms of whole animal and cellular CO₂ chemosensitivity against a background of acute and chronic oxidative and nitrosative conditioning to determine the role of redox signaling in CO₂ chemoreception (19). For example, the ventilatory response or cellular response to hypercapnic acidosis and metabolic acidosis could be studied by employing sustained hyperoxia to induce ROS/RNS production. These studies, of course, will need to redefine the control O₂ conditions, which are usually hyperoxic (see below). Alternatively, dietary manipulations that upregulate or downregulate endogenous antioxidant defenses can be employed, or animal models lacking the requisite oxidative enzymes for producing ROS and RNS (e.g., SOD, NOS knockout mice, etc.).

OXYGEN AND ACIDOSIS STIMULATE SC NEURONS

If neurons in CO₂-chemosensitive areas of the brain stem employ redox signaling mechanisms, then oxidative stress is anticipated to perturb neuronal excitability, pH regulation, and alveolar ventilation. The evidence to date indicates that putative CO₂-chemoreceptor neurons in the SC are stimulated and acidified by oxidative stress (57, 58). Other chemosensitive areas have not yet been studied. In addition, hyperoxia, particularly isocapnic hyperoxia, is a powerful oxidant and stimulus of breathing, even following carotid body denervation, which indicates that hyperoxic stimuli—ROS/RNS—are acting centrally. The significance of this so called “hyperoxic hyperventilation” as evidence of redox modulation of respiratory networks was reviewed previously in Journal of Applied Physiology (19). To date, three sources of oxidative stress have been tested on neurons in the SC in rat brain stem slices: 1) hyperoxia, which increases •O₂⁻, •NO, and ONOO⁻; 2) oxidizing agents that target the amino acids cysteine and methionine to form disulfides; and 3) chemical sources of ROS (•O₂⁻ and H₂O₂) at constant hyperoxia (95%O₂).

Hyperoxia and chemical oxidants. Molecular oxygen has a positive redox potential (E = +0.82 V) making it an excellent oxidant that readily accepts electrons. Accordingly, hyperoxia is a reliable test stimulus for activating redox signaling mechanisms, especially those involving •O₂⁻, •NO, and ONOO⁻ (16–18, 20). Essentially all studies of neurons in reduced tissue preparations—such as rat brain stem slices—are done under hyperoxic control conditions (95%O₂ in CO₂/HCO₃⁻ medium). The only way to increase neural tissue partial pressure of oxygen (P O₂) further is to increase barometric pressure and use of hyperbaric oxygen (HBO₂), which is the experimental approach we have used to investigate the effects of oxidative stress on CO₂-sensitive neurons in SC (18, 19, 57). Hyperbaric O₂ decreased membrane conductance and stimulated integrated firing rate (IFR) in 38% (n = 43/113) of the SC neurons tested. Of this group, 60 neurons, which included HBO₂-sensitive and -insensitive neurons, were also exposed to hypercapnic acidosis and/or chemical oxidants. Of these neurons, 48% (n = 29/60) were stimulated by HBO₂ and/or chemical oxidants; and of these, 90% (n = 26/29) were CO₂ excited. The excitatory effects of HBO₂ were blocked by the antioxidant, Trolox C, a membrane-permeable vitamin E analog. The excitatory effects of HBO₂ were mimicked by application of a chemical oxidant in 95% O₂ (57). Two chemical oxidants were tested, chloramine-T (CT) and N-chlorosuccinimide (NCS), neither of which produces ROS/RNS, but both are fairly specific oxidizers of cysteine and methionine. These chemical oxidants stimulated both CO₂-excited neurons and CO₂-insensitive neurons; altogether, 63% of the SC neurons tested were stimulated by CT/NCS. Whenever high CO₂ and HBO₂ were coadministered, the combined stimulation was greater than either stimulus alone. In half the neurons tested, the IFR response to hyperoxia and hypercapnia was additive, whereas in the other half of neurons tested the combined IFR response to hyperoxia and hypercapnia was greater than the sum of the individual responses.
The unspoken caveat in brain slice studies is that equilibrating nutrient medium with 95% O₂ produces tissue hyperoxia throughout a 300- to 400-μm slice, including the core, that is equivalent to a rat breathing >2 atm of HBO₂ (18). Hyperoxic control medium induces ROS production in brain slices and increases cell death (12, 16, 17). Given that HBO₂ stimulates certain SC neurons maintained in 95% O₂ at room pressure (57), and that[^1] O₂ production increases significantly in brain slices maintained in 95% O₂ for 4 h (12, 16, 17), we have initiated experiments using a lower control level of O₂ (20–40%). Under these conditions, SC neurons in 300- to 400-μm-thick transverse slices, harvested from neonates and weaned rats, remained viable for many hours based on electrophysiology criteria (50) and capacity for cells to produce ROS during O₂ manipulation (16, 17). Under these new control conditions, acute exposure to normobaric hyperoxia (95% O₂) also stimulated IFR neurons in the SC, including CO₂-excited and CO₂-inhibited neurons (50).

**Chemical oxidants, ROS, and intracellular pH.** Acute exposure to chemical oxidants and generators of ROS in 95% O₂ caused significant decreases in pHi in SC neurons. Chemical oxidants CT and NCS significantly inhibited Na⁺/H⁺ exchange leading to intracellular acidosis. Likewise, H₂O₂ and dihydroxyfumarate, which generates extracellular •O₂, acidified SC neurons (58). The effect of normobaric hyperoxia on pHi, by comparison, is more complicated than the other oxidative stimuli tested since O₂ availability influences a broader range of redox reactions through simultaneous production of •O₂ and •NO and activation of multiple downstream redox reactions. In addition, hyperoxia will affect cellular respiration and neuronal activity. All of these effects, in turn, will influence pHi. In SC neurons, pHi is more alkaline during normobaric hyperoxia and decreases with decreasing tissue PO₂. Specifically, average pHi plateaus at 7.44–7.45 in superfusate equilibrated with 80–95% O₂. Decreasing steady-state O₂ to 60–40% decreases pHi to another plateau averaging 7.34–7.35 (69). These data suggest that the excitatory effect of hyperoxia on the IFR of SC neurons (57) is mediated primarily by cellular oxidation rather than hyperoxia-induced acidification. At constant O₂, however, conditions that increase production of ROS and cause cellular oxidation would both acidify and oxidize SC neurons and IFR would be determined by the balance between oxidation and acidification (58), as illustrated next.

In the case of CT and NCS, one must consider the effects of both cellular oxidation and intracellular acidosis; that is, because chemical oxidants applied at constant O₂ resulted in decreased pHi, were SC neurons stimulated by intracellular acidosis caused by oxidative stress or was the effect of cellular oxidation separate from that of cellular acidosis? To determine this, Mulkey et al. (58) developed a novel method for clamping pHi at control level during exposure to CT using CO₂/HCO₃⁻ manipulations. These experiments showed that SC neurons increased IFR in the absence of intracellular acidosis indicating cellular oxidation alone stimulated SC neurons. Exposure to simultaneous cellular oxidation and acidification tended to cause a larger stimulation of IFR (58).

To summarize the final section, the foregoing discussion indicates that neurons in the dorsomedial chemosensitive area of the medulla oblongata are stimulated by various sources of oxidative stimuli under in vitro conditions. The excitatory effects of hyperoxia are caused by decreased membrane conductance and are reversibly blocked by a membrane-permeable antioxidant. Putative CO₂-chemoreceptor neurons, that is, neurons stimulated by hypercapnic acidosis, are likewise stimulated by HBO₂ and chemical oxidants. Exposure to hypercapnic HBO₂ caused a greater stimulation of IFR than exposure to either stimulus alone. Chemical oxidants and generators of ROS at constant O₂ acidify SC neurons by inhibition of Na⁺/H⁺ exchange and stimulate IFR. The excitatory effects of oxidation and acidification occur independently of each other. While the stimulatory effects of hyperoxia on IFR occur primarily in chemosensitive SC neurons, the effects of chemical oxidants (at constant O₂) on IFR and pHi regulation indicate that redox stimulation influences the functions of a larger population of SC neurons. The functional significance of sensitivity to hyperoxia and chemical oxidants suggests that certain neurons in the SC, including CO₂-excited neurons (i.e., putative CO₂ chemoreceptors), use redox signaling mechanisms to carry out certain aspects of their normal functions (19). The excitability of SC neurons is determined, we propose, by the relative levels of pHi/pH₀ and ROS/RNS at any given time (58), both of which decrease membrane conductance. Presumably, different populations of pH-sensitive channels and redox-sensitive channels are involved, which include potassium channels (57, 58, 72). Redox- and nitrosative-sensitive components of the plasma membrane that are targeted by ROS and RNS during hyperoxia likewise are affected, we hypothesize, by ROS and RNS generated during hypercapnic acidosis (via CO₂/ONOO⁻ reaction) or isocapnic acidosis at constant O₂ (via Fenton reaction) (Fig. 1). These same redox sensitive mechanisms would also render neurons in the SC vulnerable to oxidative and nitrosative stress during pathological conditions of ischemia, hyperoxia, and chronic respiratory acidosis and metabolic acidosis.

**Perspectives**

Carbon dioxide, like O₂ and several other gas species (70), is able to effect redox signaling mechanisms through production of oxidative and nitrosative derivatives. These effects of hypercapnia and acidosis on redox signaling have rarely been considered in studies of central CO₂ chemoreception (19, 57). During CO₂ retention, therefore, the scope of their importance in defining brain stem activity and, ultimately, alveolar ventilation, is unknown and requires further research. The ventilatory response in the conscious animal to changes in CO₂/pH is incredibly sensitive (34, 59, 81). I am postulating that these CO₂/H⁺-sensitive redox reactions are poised, under physiological conditions, such that small perturbations in CO₂/pH produce changes in redox state that, in tandem with changes in cellular pH, affect central chemoreceptors (and other cardiopulmonary neurons) and thus alveolar ventilation. Clearly, the data indicate that neurons in multiple chemosensitive areas express the biochemical machinery for producing significant quantities of ROS/RNS during hypercapnic acidosis and isocapnic acidosis. Moreover, neurons in at least one chemosensitive area (SC) are stimulated directly by CO₂-induced acidification and oxidation, and indirectly through oxidation-induced acidification (57, 58). Thus chemoreceptor activity, at least in the SC, is going to be determined by the balance between the relative levels of pHi and redox state during hypercapnic acidosis (58). Furthermore, the influence of any
CO2/H+–driven redox reaction will be amplified by conditions that enhance production of their reactants; that is, •O2 and •NO for the CO2/ONOO– reaction, and •O2, iron, and H2O2 for the Fenton reaction. Hence, hypoxia, posthypoxic reoxygenation, and hyperoxia (17), when combined with CO2 retention, will result in increased ROS/RNS production through the combined redox and nitrosative chemistry of molecular O2 and CO2/H+.

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REFERENCES

25. Faith CH, Chan JYH, Chan SHH, Chang AYW. In the rostral ventrolateral medulla, the 70-kDa heat shock protein (HSP70), but not HSP90, confers neuroprotection against fatal endotoxemia via augmentation of nitric-oxide synthase I (NOS 1)/protein G signaling pathway and inhibition of NOS II/peroxynitrite cascade. Mol Pharmacol 68: 179–192, 2005.


80. Simpson KL, Waterhouse BD, Lin RCS. Differential expression of nitric oxide in serotoninergic projection neurons: neurochemical identification...


