Oxidative stress decreases pH$_i$ and Na$^+$/H$^+$ exchange and increases excitability of solitary complex neurons from rat brain slices

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METHODS

Brain slice. Details regarding the preparation of brain slices have been described elsewhere (14). Briefly, a brain stem from a rat pup ranging in postnatal (P) age from P1 to P15 days was isolated, and the medulla oblongata was cut into 300-μm-thick transverse slices by using the Vibratome (series 1000) sectioning system. Slices were taken beginning at obex and moving rostrally for ∼900 μm. Animal use procedures are in agreement with the Wright State University Institutional Animal Care and Use Committee guidelines and were approved by the committee (AALAC no. A3632-01). Brain stem slices were incubated at room temperature in artificial cerebral spinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 5.0 KCl, 1.3 MgSO$_4$, 26 NaHCO$_3$, 1.24 KH$_2$PO$_4$, 2.4 CaCl$_2$, and 10 glucose, equilibrated with 95% O$_2$/5% CO$_2$ (P$_{CO2}$ ∼720 Torr, P$_{O2}$ ∼40 Torr) (36). Under these conditions, slices remained viable for electrophysiological experiments for ∼8 h.

pH$_i$ measurements. We measured pH$_i$ by using ratiometric fluorescence imaging microscopy, utilizing either the pH-sensitive fluorescent dye BCECF or, during whole cell recordings, pyranine in SC neurons in brain stem slices from rat pups. Oxidative stress decreased pH$_i$ in 270 of 436 (62%) SC neurons tested. Chloramine-T (CT), N-chlorosuccinimide (NCS), dihydroxyfumaric acid, and H$_2$O$_2$ decreased pH$_i$ by 0.19 ± 0.007, 0.20 ± 0.015, 0.15 ± 0.013, and 0.08 ± 0.002 pH unit, respectively. Hypercapnia decreased pH$_i$, by 0.26 ± 0.006 pH unit (n = 95). The combination of hypercapnia and CT or NCS had an additive effect on pH$_i$, causing a 0.42 ± 0.03 (n = 21) pH unit acidification. CT slowed pH$_i$ recovery mediated by Na$^+$/H$^+$ exchange (NHE) from NH$_4$Cl-induced acidification by 53% (n = 20) in CO$_2$/HCO$_3$-buffered medium and by 58% (n = 10) in HEPES-buffered medium. CT increased firing rate in 14 of 16 SC neurons, and there was no difference in the firing rate response to CT with or without a corresponding change in pH$_i$. These results indicate that oxidative stress 1) decreases pH$_i$ in some SC neurons, 2) together with hypercapnia has an additive effect on pH$_i$, 3) partially inhibits NHE, and 4) directly affects excitability of CO$_2$/H$^+$-chemosensitive SC neurons independently of pH$_i$ changes. These findings suggest that oxidative stress acidifies SC neurons in part by inhibiting NHE, and this acidification may contribute ultimately to respiratory control dysfunction.

hyperoxic hyperventilation; O$_2$ toxicity; pH regulation; brain stem; reactive oxygen species

CO$_2$/H$^+$-chemosensitive neurons, which are believed to function as CO$_2$ chemoreceptors for the cardiorespiratory control system, are highly sensitive to oxidative stress. For example, we found that CO$_2$/H$^+$-chemosensitive, but not CO$_2$/H$^+$-insensitive, SC neurons are stimulated by hyperoxia (38). We went on to show that the responses to CO$_2$ and hyperoxia are mediated by separate mechanisms; the CO$_2$ signal involves decreased intracellular pH (pH$_i$) (21) whereas hyperoxia depends on oxidation (38). However, when SC neurons were exposed to CO$_2$ and hyperoxia in combination, the firing rate response was larger than to either stimulus alone (38), thus suggesting that oxidative stress and CO$_2$ signaling mechanisms are at least additive in SC neurons.
BCECF is in a state that is not likely to be further oxidized by the level of oxidative stress used in this study (54). Therefore, we assumed that oxidative stress in the form of either chemical oxidants or free radicals would not have any direct effect on the fluorescence of the dye. Details regarding the use of BCECF for pH imaging in brain slices have been previously described (49, 50). Slices were loaded in the dark with 20 μM BCECF (in the membrane-permeable acetoxymethyl ester form) for 30–60 min at 37°C and washed at room temperature in control aCSF (50). Experiments were performed on individual slices transferred to a superfusion chamber positioned on the stage of an inverted Nikon Diaphot microscope where the perfused water aCSF, heated to 37°C (pH ~7.45), at 2 ml/min. The dye was excited every 60 s by brief (~1 s) alternating pulses of light with wavelengths of 500 nm (pH sensitive) and 440 nm (pH insensitive). pH is proportional to the ratio of emitted fluorescence (535 nm) at these two excitation wavelengths (F_{so}/F_{440}). This fluorescence ratio was normalized to the fluorescence ratio value at pH 7.2, determined by using the high-K+/nigericin calibration technique (62), and normalized fluorescence was converted to pH, by using the equation of Ritucci et al. (49, 50). To minimize any effects from nigericin contamination, we used a separate calibration line (distinct from the perfusion lines), and the tissue chamber was extensively washed following each experiment.

In a second series of experiments, we measured pH by using the pH-sensitive fluorescent dye pyranine while simultaneously measuring membrane potential (V_m) with the use of whole cell recording (WCR) techniques. Pyranine was used in these experiments because 1) it is not membrane permeable and so can only be loaded into neurons by WCR where it tends not to leak out; 2) it is resistant to photobleaching; 3) it has a high quantum yield; and 4) it is less damaging to the cell than BCECF during excitation (23). Pyranine (300 μM) was dissolved in the whole cell solution, which diffused into the cell body only after the patch was ruptured and a WCR established. The dye was excited every 60 s by brief (~2 s) alternating pulses of light with wavelengths of 450 nm (pH sensitive) and 415 nm (pH insensitive). pH is proportional to the ratio of emitted fluorescence (515 nm) at these two excitation wavelengths (F_{so}/F_{415}). This fluorescence ratio was recorded by using MetaFluor software, normalized to the fluorescence ratio value at pH 7.2, and then normalized fluorescence was converted to pH by using the equation of Ritucci et al. (51).

Electrophysiology. Loose extracellular patches and WCR were made by using the Axopatch-1D (CV-4 head-stage gain = 1.0; Axon Instruments), as described previously (30, 42). Briefly, the recording electrode was made from borosilicate glass (TW150-3; World Precision Instruments) by using a two-stage Narishige pipette puller (PP-830). The electrode was filled with a solution containing (in mM) 130 K-glucuronate, 0.4 EGTA, 1.0 MgCl₂, 0.3 Na₂-GTP, 2.0 Na₂-ATP, and 10 HEPES, pH 7.45 at room temperature. Compared with standard filling solution, this solution had reduced EGTA and no CaCl₂ to reduce washout (22). The recording electrode was connected to the head stage by a Ag-AgCl wire (Medwire), and an AgCl reference was placed in the tissue bath to complete the circuit. Recording electrodes had a tip resistance of ~3 MΩ; electrodes with smaller tips (tip resistance > 5 MΩ) were not used because they are not conducive to loose patch recording (42). The recording pipette and SC neurons were visualized (×720 magnification) with an Optiphot-2 Nikon microscope with a ×40 water-immersion Hoffman contrast objective (NA 0.55). A slight positive pressure was constantly applied to the electrode to keep the seal from leaking.

Analysis and data presentation. Data were analyzed by using AxiScope 8.1, SigmaPlot, Origin 5.0, and SigmaStat software packages. The CoreDraw 8.0 software package was used for data presentation. To determine whether a test condition caused a change of pH, a change of >0.05 pH unit was defined as significant. Paired-sample t-tests (P < 0.05 unless otherwise stated) were used to determine when the average change in the pHi firing rate, or rate of pH recovery differed significantly from zero. Significant differences among the effects of each test condition on pHi, or firing rate were determined by using a one-way ANOVA and Newman-Keuls multiple comparison test (P < 0.05) when appropriate. All results were presented as means ± SE.

RESULTS

The experiments in this study were conducted in two parts. First, we measured pHi to determine whether oxidative stress affects pHi or pH regulation. In the second part of this study, we combined WCR of neuronal excitability with pHi measured with pyranine to determine whether oxidative stress stimulates firing rate independently of changes in pHi.

Effects of oxidative stress on pHi. pH measured with BCECF in slices incubated in control aCSF was 7.40 ± 0.005 (n = 95) and was similar to values previously reported for SC neurons (49). Exposure to oxidative stress significantly decreased pHi in many SC neurons (Fig. 1). In fact, oxidative stress resulted in acidification of 62% (270 of 436) of SC neurons tested. The chemical oxidants CT and NCS decreased pHi by 0.19 ± 0.007 (n = 111) and 0.20 ± 0.015 (n = 30), all of which is also known to stimulate the firing rate of SC neurons (38, 53). Dithiothreitol (DTT; 0.5–1 mM), a hydrophilic and cell-permeable cysteine-specific reducing agent (20), was used to differentiate the effects of CT- and NCS-induced oxidation of cysteine vs. methionine. All test solutions had a pH of 7.45 after equilibration with 5% CO₂–balance O₂ at 37°C. Finally, to determine the relative effect of oxidative stress in the absence of a change in pHi, we blocked CT-induced acidification by either increasing NaCl to 98 mM or lowering CO₂ from 5 to 1.4%.

pHi regulation. NHE activity was determined by measuring pH recovery from an ammonium chloride (NH₄Cl; 20 mM)-induced intracellular acidification (osmolality was maintained by decreasing NaCl to 104 mM) under control conditions and during oxidative stress imposed by CT. To determine whether our control conditions (CO₂/HCO₃ buffering system) unexpectedly contributed to the effects of oxidative stress on NHE activity, we also measured the effects of CT on pHi recovery in HEPES-buffered medium of the following composition (in mM): 124 NaCl, 5.0 KCl, 1.3 MgSO₄, 26 HEPES, 1.24 KH₂PO₄, 2.4 CaCl₂, and 10 glucose, pH 7.4 at 37°C, equilibrated with 100% O₂. Amiloride (1.6 mM) was used to block NHE.
Mide (NCS; 1 mM) decreased pHi. In some cells, the effects of CT on pHi were greater than those induced by either DHF (DHF; 3 mM) or H2O2 (H2O2; 4–5 mM). This suggests that cysteine-specific oxidation is critical for CT- and NCS-induced acidification. Conversely, if DTT did not reverse the effects of these oxidants, it would suggest that methionine oxidation is critical for CT- and NCS-induced acidification. As shown in Fig. 1C, at a 1:1 (n = 20) or 2:1 (n = 11) ratio of oxidant to reducing agent, DTT did not reverse the acidification caused by CT (n = 28) or NCS (n = 3). That is, the change in pHi caused by CT (Fig. 1Ai) or NCS (Fig. 1A) remained significantly different from control during 10-min recovery in control medium (ΔpHi = 0.23 ± 0.02, n = 30) and after 10-min exposure to DTT (ΔpHi = 0.21 ± 0.02, n = 30) (Fig. 1C). Exposure to DTT alone had no effect on pHi (data not shown). These results suggest that oxidation of the amino acid methionine plays an important role in CT- and NCS-induced acidification.

The remaining 38% (166 of 436) of SC neurons showed no significant change in pHi (i.e., ≤ 0.05 pH unit) during exposure to chemical oxidants or ROS (Fig. 2). The population of insensitive neurons included neurons whose pHi did not respond to NCS (n = 23) (Fig. 2Aa), CT (n = 12) (Fig. 2Ab), DHF (n = 31), and H2O2 (n = 100) (Fig. 2B). The neurons that did not respond with a change in pHi during oxidative stress appeared to be healthy (strong fluorescence ratio) and were qualitatively similar in size and morphology to cells that were acidified by oxidative stress. In addition, these insensitive neurons exhibited a normal pHi response to hypercapnic acidification.

To determine whether the effects of CT or NCS are mediated by the differential oxidation of cysteine or methionine, we attempted to reverse the effects of CT- and NCS- induced acidification with the cysteine-specific reducing agent DTT. If DTT were to reverse the effects of CT or NCS, it would suggest that cystein-specific oxidation is critical for CT- and NCS-induced acidification. Conversely, if DTT did not reverse the effects of these oxidants, it would suggest that methionine oxidation is critical for CT- and NCS-induced acidification.

Fig. 1. Exposure to oxidative stress decreased intracellular pH (pHi) in 62% of solitary complex (SC) neurons. A: pHi traces show that exposure (5–15 min) to the chemical oxidants chloramine-T (CT; 500 μM) and N-chlorosuccinimide (NCS; 1 mM) decreased pHi. In some cells, the effects of CT on pHi were fully reversible (i); however, more typically, chemical oxidants induced an acidification that was only partially reversible (ii; CT, NCS). B: pHi traces show that exposure (5–15 min) to superoxide (O2••) produced from dihydroxyfumaric acid (DHF; 3 mM), or hydrogen peroxide (H2O2; 4–5 mM) reversibly decreased pHi. C: pHi traces show that exposure to CT (500 μM) or NCS (1 mM) caused a sustained acidification and that application of DTT (1 mM) either during (not shown) or 10 min after exposure to CT or NCS had no effect on pHi recovery. D: Bar graph showing the magnitude of the acidification (ΔpHi) caused by each oxidant used. All ΔpHi were significantly different from zero (P < 0.001). *ΔpHi differed significantly from H2O2; **ΔpHi differed significantly from DHF and H2O2. DTT did not alter the acidification caused by CT or NCS (P > 0.05).

respectively (Fig. 1, A and D). Likewise, the free radical O2••, produced by DHF, as well as the reactive nonradical H2O2, decreased pHi by 0.15 ± 0.013 (n = 17) and 0.08 ± 0.002 (n = 112), respectively (Fig. 1, B and D). Acidifications induced by 4 and 5 mM H2O2 were not significantly different and were therefore pooled. Note that both O2•• and H2O2 are considered ROS. CT- and NCS-induced acidifications were of a larger magnitude than those induced by either DHF (P < 0.05) or H2O2 (P < 0.001), and H2O2 had the smallest effect on pHi (Fig. 1D).

Although both forms of oxidative stress (i.e., chemical oxidants and ROS) decreased pHi to a stable plateau rather than causing a continued acidification during oxidative stress, the acidifications induced by CT and NCS were less reversible than those induced by DHF or H2O2 (Fig. 1, compare A and B). This finding suggests that CT and NCS had a stronger effect on pHi than did DHF or H2O2. This possibility is further supported by the finding that a greater proportion of neurons responded with a change in pHi to CT and NCS (80%) than responded to DHF or H2O2 (50%).

To determine whether the effects of CT or NCS are mediated by the differential oxidation of cysteine or methionine, we attempted to reverse the effects of CT- and NCS- induced acidification with the cysteine-specific reducing agent DTT. If DTT were to reverse the effects of CT or NCS, it would suggest that cystein-specific oxidation is critical for CT- and NCS-induced acidification. Conversely, if DTT did not reverse the effects of these oxidants, it would suggest that methionine oxidation is critical for CT- and NCS-induced acidification.
dosis (Fig. 2). Together, these results indicate that the levels and exposure times of chemical oxidants and ROS used decreased the pH of many, but not all, SC neurons.

**Oxidative stress slows pHi recovery from acidification.** To determine whether NHE activity is slowed by oxidative stress, we measured pH i recovery from an NH4Cl-induced acidification under control conditions and in the presence of CT. We have previously shown (48) that SC neurons regulate pH i from acidification by the activity of NHE only. Therefore, the rate of pH i recovery from an intracellular acidification is assumed to be directly proportional to the activity of NHE.

We found that NHE activity was slowed by oxidative stress. In CO2/HCO3−-buffered medium, pH i recovery from an ~0.3 pH unit acidification, to a minimum pH i of 7.11 ± 0.02 (Fig. 3A, first NH4Cl prepulse), occurred at a rate of 0.017 ± 0.002 pH unit/min. pH i recovery of the same neurons to a second ~0.3 pH unit acidification, to a minimum pH i of 6.83 ± 0.12, was slowed 47% by CT (Fig. 3A, second NH4Cl prepulse). Note that in these experiments, NH4Cl-induced acidification during oxidative stress decreased pH i to a more acidic minimum value. Activity of NHE is known to be inversely proportional to pH i; high pH i values inhibit NHE activity, whereas lower pH i values increase NHE activity (43). Therefore, on the basis of the more acidic minimum pH i value, we would expect higher NHE activity in the presence of CT than in its absence. We observed just the opposite, suggesting that CT inhibits NHE and that we are likely underestimating the inhibitory effect of oxidative stress on NHE activity at the lower initial pH i values.

To better determine the effects of oxidative stress on NHE, we measured pH i recovery in the presence and absence of CT under conditions of similar acidification and with minimal buffering power (nominal absence of CO2/HCO3−). In HEPES-buffered medium, recovery from acidification to a similar minimum pH i value (6.83 ± 0.12; n = 10) was slowed by 43% by CT (Fig. 3B). The effects of CT on pH i recovery and NHE activity are summarized in Fig. 3C. These results clearly demonstrate that NHE activity was significantly slowed by the chemical oxidant CT.

**Combined effects of hypercapnia and chemical oxidants on pH i.** Oxidative stress and hypercapnia both cause a fall of pH i (Figs. 1–3), raising the possibility that the effects of hypercapnia and oxidative stress on pH i are additive. Therefore, we wanted to determine whether neurons could respond to hypercapnia during oxidative stress. We found that exposure to 15% CO2 decreased pH i from a control level of 7.40 ± 0.005 by 0.26 ± 0.006 pH units (n = 95) (Fig. 4). Exposure to the chemical oxidants CT or NCS, as previously mentioned, decreased pH i from 7.32 ± 0.01 by 0.19 ± 0.007 pH units (n = 111) or 0.20 ± 0.015 pH units (n = 30) (Fig. 4). In the presence of CT or NCS, exposure to 15% CO2 decreased pH i by 0.26 ± 0.014 pH units (n = 21), which resulted in a total change in pH i of 0.42 ± 0.003 pH unit from control level (Fig. 4, A and B). Therefore, exposure to 15% CO2 plus oxidant caused a significantly greater fall of pH i than exposure to either 15% CO2 or oxidant alone (Fig. 4C), indicating that the effects of oxidative stress and hypercapnia on pH i are additive.

**Oxidative stress without a change of pH i.** Recently, we showed (38) that oxidative stress, including CT, stimulates firing rate of CO2/H+−chemosensitive SC neurons. These same putative CO2/H+ chemoreceptors also respond to hypercapnia.
Fig. 4. Hypercapnia plus oxidative stress decreases pH, by more than either stimulus alone in SC neurons. A: pH trace shows that 15% CO₂ caused a ~0.3 pH unit acidification. Exposure to CT (500 µM) decreased pH, by ~0.25 pH units. During exposure to CT, the addition of hypercapnia decreased pH, by ~0.4 pH unit acidification. Exposure to NCS (1 mM) decreased pH, by ~0.25 pH units. During exposure to NCS, the addition of 15% CO₂ decreased pH, by ~0.25 pH units. B: pH trace shows that hypercapnia caused a ~0.4 pH unit acidification. During exposure to CT (500 µM) decreased pH, by ~0.2 pH units. During exposure to CT, the addition of hypercapnia decreased pH, by ~0.4 pH units. C: bar graph summarizing the effects of hypercapnia, CT or NCS (CT/NCS), and 15% CO₂ plus CT/NCS on pH. Exposure to CO₂ and chemical oxidant in combination decreased pH, significantly (**P < 0.001) more than either stimulus alone.

via a mechanism that involves decreased pH, (53). We have shown here that CT decreases pH, (Figs. 1, 3, and 4). Together, these results suggest that oxidant-induced acidification could contribute to the activation of CO₂/H⁺-chemosensitive neurons by oxidative stress. Therefore, to determine whether the firing rate response to CT is mediated by direct ion channel oxidation or indirectly via an oxidant-induced acidification, we attempted to separate the oxidant and pH, effects by blocking the acidification caused by CT with a comparable and simultaneous alkalinization. Two approaches were employed. We made a series of pH measurements using BCECF while increasing HCO₃⁻ concentration or decreasing Pco₂ of the aCSF to determine what level of HCO₃⁻ or CO₂ causes an ~0.2 pH unit alkalization. Increasing the concentration of HCO₃⁻ from 26 to 52 mM increased pH, by ~0.2 pH units (Fig. 5Aa) (48). The time course of this alkalinization was similar to the time course of the acidification caused by CT (Figs. 1, 3, and 4); i.e., the initial change in pH, occurred within ~3 min and reached a maximum in ~5 min (Fig. 5Aa). Therefore, we attempted to block CT-induced acidification by exposing SC neurons to CT and 52 mM HCO₃⁻ at the same time. Simultaneous exposure to CT plus 52 mM HCO₃⁻ resulted in no significant change of pH, (0.019 ± 0.012 pH units; n = 17) (Fig. 5, B and D), indicating that we were able to block the CT-induced acidification using high HCO₃⁻.

Alternatively, CT-induced acidification was blocked by keeping HCO₃⁻ constant at 26 mM, and the CO₂ level was decreased from 5 to 1.4%. This approach was used so that HCO₃⁻ could be held constant, because a previous study showed that varying the bicarbonate concentration can affect neuronal excitability (5). Lowering CO₂ from 5 to 1.4% caused a 0.19 ± 0.024 (n = 10) pH unit alkalinization (Fig. 5Ab). This hypocapnic alkalinization was of a similar magnitude to the acidification caused by CT (Figs. 1, 3, and 4); however, changing CO₂ affected pH, much more rapidly than CT did (Fig. 5Ab). Therefore, to minimize any change in pH, with the use of this paradigm, exposure to hypocapnic solution was
initiated ~3 min after initiation of exposure to CT. This procedure resulted in no significant CT-induced change in pHi (0.027 ± 0.09 pH units; n = 36) (Fig. 5, C and D). Thus we were able to block CT-induced acidification by using hypocapnic solutions. Furthermore, either high-HCO₃⁻ or hypocapnic solutions were equally effective at blocking the acidification caused by CT (ANOVA, P > 0.05) (Fig. 5D). These experiments indicate that we are able to expose SC neurons to oxidative stress imposed by CT in the absence of any change in pHi.

Oxidative stress and decreased pHi stimulate SC neurons.

The next goal of this study was to measure the effects of hypercapnia and oxidative stress on firing rate and pHi, simultaneously, of SC neurons. To make this determination, WCRs of Vm were made to measure firing rate while pHi was simultaneously measured by using ratiometric fluorescence imaging microscopy with the pH-sensitive dye pyranine. As shown in Fig. 6A, when the cell membrane was ruptured to establish an intracellular recording, pyranine in the pipette filling solution rapidly diffused into the cell. The time constant for loading an SC neuron with pyranine with a WCR pipette was determined by fitting fluorescence intensity over time to the curve

\[ Y = \frac{F_{\text{max}}(x)}{x + t_k} \]

where \(F_{\text{max}}\) is maximum fluorescence intensity obtained at the pH-insensitive wavelength 415 nm, \(x\) is time, and \(k\) is one-half \(F_{\text{max}}\). The time required to reach \(k\), defined as the time constant \((t_k)\) for pyranine loading, was 214 ± 63 s (n = 5) (Fig. 6B). Therefore, using the whole cell configuration, we were able to rapidly load individual neurons with pyranine. However, diffusion of pipette solution into the cell also causes the dilution or washout of soluble intracellular material (28), including potentially vital components of the CO₂/H⁺-chemosensitive signaling mechanism. For example, previous studies have shown that CO₂/H⁺ chemosensitivity is highly susceptible to washout during WCR (15, 47).

CT sensitivity of SC neurons.

In the whole cell configuration, exposure to CT increased firing rate on average by 3.13 ± 0.8 impulses/s from an initial firing rate of 0.71 ± 0.38 impulses/s with a corresponding decrease of pHi of ~0.2 pH units in 15 of 18 (83%) SC neurons. Two of three SC neurons that did not respond to CT with a change in firing rate also did not show a change of pHi during exposure to CT (not shown), thus
suggesting that oxidant-induced acidification contributes to the effect of CT on neuronal excitability.

To determine whether SC neurons can respond to CT despite washout of CO\textsubscript{2}/H\textsuperscript{+} chemosensitivity, we exposed a cell to 15% CO\textsubscript{2} while recording single-cell activity extracellularly in the loose patch configuration. The loose patch recording in Fig. 7A shows that exposure to 15% CO\textsubscript{2} increased the firing rate of the neuron. After the patch was ruptured for intracellular recording and \~10 min were allowed for pyranine to fully diffuse into the cell (Fig. 6, A and B), a second exposure to 15% CO\textsubscript{2} did not increase the firing rate of the neuron. One concern was that this neuron’s spontaneous firing rate ceased during WCR because of a slow hyperpolarizing drift, a phenomenon that has been previously observed (22). To assure that this hyperpolarization was not the basis for loss of CO\textsubscript{2}/H\textsuperscript{+} chemosensitivity, we injected small subthreshold depolarizing pulses to bring V\textsubscript{m} close to spike threshold. Regardless, hypercapnia was still not able to stimulate firing rate. This washout of the firing rate response to hypercapnia cannot be due to clamping of pH\textsubscript{i} by the whole cell solution because hypercapnia resulted in a large and maintained acidification of the neuron (Fig. 7B). These results indicate that the firing rate response, but not the pH\textsubscript{i} response, to hypercapnia had been washed out during WCR. In the absence of a functional CO\textsubscript{2}/H\textsuperscript{+}-chemosensing mechanism, exposure to CT decreased pH\textsubscript{i} by \~0.13 pH units and increased the firing rate of the same SC neuron (Fig. 7B). These results indicate that chemosensitive SC neurons can respond to CT with an increase in firing rate independently of changes of pH\textsubscript{i}. These results support our hypothesis that the oxidant and pH\textsubscript{i} signaling mechanisms are functionally distinct.

Oxidant stimulation of firing rate occurs independently of changes of pH\textsubscript{i}. Exposure to CT increased firing rate with a corresponding decrease in pH\textsubscript{i} (Figs. 7B and 8A). When the CT-induced acidification was blocked with either the high-HCO\textsubscript{3}\textsuperscript{-} or hypocapnic CT exposure paradigms described above, the oxidant still stimulated firing rate in 14 of 16 (88%) SC neurons tested. For example, exposure of an SC neuron to CT in high-HCO\textsubscript{3}\textsuperscript{-} solution increased firing rate without a change of pH\textsubscript{i} (Fig. 8B). Likewise, exposure of an SC neuron to CT in hypocapnic solution resulted in an increase of firing rate with no change in pH\textsubscript{i} (Fig. 8C). These results indicate that the oxidative stress caused by CT can stimulate firing rate independently of changes of pH\textsubscript{i}.

Contribution of pH\textsubscript{i} to the oxidant-induced stimulation of SC neurons. CO\textsubscript{2}/H\textsuperscript{+}-chemosensitive neurons, like the ones that we are studying, are stimulated by decreased pH\textsubscript{i} (21, 53). Therefore, we wanted to quantify the contribution of the oxidant-induced acidification to the CT-induced increase in firing rate of SC neurons. To make this determination, we compared the firing rate response of neurons exposed to CT with a corresponding acidosis (e.g., Figs. 7B and 8A) to the firing rate response evoked by CT in the presence of high-HCO\textsubscript{3}\textsuperscript{-} or hypocapnic solutions (e.g., Figs. 8, B and C). As shown in Fig. 9A, all three conditions, CT and CT with either high HCO\textsubscript{3}\textsuperscript{-} or low CO\textsubscript{2}, were able to stimulate firing rate in the same neuron. However, neurons were typically not exposed to multiple bouts of CT (with or without a corresponding acidosis) because the effects of CT on firing rate tended to be poorly reversible and WCRs frequently became unstable after exposure to CT. Therefore, comparisons were made between populations of neurons exposed to CT alone, CT in the presence of high-HCO\textsubscript{3}\textsuperscript{-}, and CT in hypocapnic solution. The firing rate response to CT and corresponding acidification was not significantly greater than the firing rate response to CT when either 52 mM HCO\textsubscript{3}\textsuperscript{-} or 1.4% CO\textsubscript{2} was used to block the CT-induced acidification (Fig. 9B). These results suggest that the effects of CT on pH\textsubscript{i} do not contribute significantly to the

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**Fig. 7.** Sensitivity of SC neurons to CT does not washout in CO\textsubscript{2}/H\textsuperscript{+}-chemosensitive SC neurons. A: in the loose patch configuration, traces of integrated firing rate (IFR) and V\textsubscript{m} show an SC neuron that is stimulated by 15% CO\textsubscript{2}. B: traces of IFR, pH\textsubscript{i} (measured by pyranine), and voltage trajectories (3 V\textsubscript{m} traces superimposed) show that after the cell membrane was ruptured for WCR, spontaneous activity was lost. A depolarizing current pulse (0.3 nA, 50 ms) was used to bring V\textsubscript{m} near action potential threshold. In this configuration, exposure to 15% CO\textsubscript{2} decreased pH\textsubscript{i} but did not result in increased firing rate. These results indicate that CO\textsubscript{2}/H\textsuperscript{+} chemosensitivity had been lost due to washout during WCR. In contrast, as shown by the traces of IFR and V\textsubscript{m}, exposure to CT did increase neuronal firing rate.
response of SC neurons to CT and, thus, that CT must have direct effects on SC neuron excitability independent of changes of pH

DISCUSSION

We have described for the first time the effects of oxidative stress on pH and NHE activity of neurons in a region of CO₂/H⁺ chemoreception for the respiratory control system (i.e., the solitary complex). Our results show that oxidative stress decreases pH in the majority of SC neurons tested and slows NHE activity. We went on to show that oxidation could activate SC neurons in a manner that is independent of changes of pH. This conclusion is supported by our findings that the chemical oxidant CT can stimulate firing rate in the absence of a change in pH, as well as when CO₂/H⁺ chemosensitivity has been washed out during WCR. These results indicate that SC neurons can respond to CT independently of changes in pH.

Oxidant-induced intracellular acidosis. Our results show that the chemical oxidants CT and NCS, O₂ produced by DHF, and H₂O₂ decrease pH in 62% of SC neurons. There were, however, differences in the magnitude of the acidification caused by each oxidant. CT and NCS caused a larger and more sustained acidification than did DHF or H₂O₂, whereas H₂O₂ evoked the smallest change in pH and constituted the largest proportion of oxidant-insensitive responses. The remaining 38% of SC neurons tested did not show a significant change in pH during exposure to oxidative stress.

The concentrations of chemical oxidants used in this study were the same as those previously shown to increase firing rate and input resistance of SC neurons, many of which were CO₂/H⁺ chemosensitive (38). The effects of CT and NCS on pH were remarkably similar to their effects on excitability of SC neurons as measured by sharp electrode recording (38). Just as with changes of pH, CT and NCS increased firing rate in about two-thirds of SC neurons tested, and their effects were not fully reversible (38). CT and NCS are thought to specifically oxidize cysteine and methionine amino acids (55), hence CT- and NCS-induced acidification likely involves the oxidation of critical methionine and/or cysteine amino acids, of which NHE has several (64). Furthermore, the acidification caused by CT and NCS was not reversed by the cysteine-specific reducing agent DTT, thus further narrowing the oxidant target to methionine. In addition, comparable to the mild effects of DHF and H₂O₂ on pH, hyperoxia (~1,600–2,500 Torr), an apparently milder and probably more general form of...
oxidative stress, increased firing rate in only 38% of SC neurons and in a reversible fashion (38). These results suggested that the effect of oxidative stress on pHᵢ contributes in part to the effects of oxidative stress on neuronal excitability of SC neurons. Therefore, we went on to test this possibility in the second part of this study by measuring Vᵢm with WCR while simultaneously measuring pHᵢ with pyranine.

Previous studies in nonneuronal preparations have shown that oxidative stress has variable effects on pHᵢ. For example, H₂O₂ caused an intracellular acidosis in rat cerebellar astrocytes (63), C6 glioma cells (63), human promyelocytic leukemia cell line HL60 (27), human aortic endothelial cells (29), OK and BSC-1 renal epithelial cells (33), and rat and human cardiac myocytes (9, 65). Likewise, O₂⁻ produced by the tert-butylhydroperoxide (t-BHP) decreased pHᵢ in Xenopus oocytes (10). Furthermore, the H₂O₂-induced acidification of myocytes was dose dependent at concentrations ranging from 30 μM to 3 mM (34). Conversely, oxidative stress has also been reported to increase pHᵢ in certain cell types. For example, oxidative stress imposed by hyperoxia or by O₂⁻ or hydroxyl radicals increased pHᵢ of U937 phagocytes (56), hepatic stellate cells (60), and canine kidney epithelial cells (17).

Together, these observations indicate that oxidative stress can affect pHᵢ. However, similar oxidants have opposite effects on different cell types, whereas similar cell types (e.g., renal epithelial cells) respond differently to different oxidants. These disparate findings suggest that the effects of oxidative stress on pHᵢ depend on both the cell type and the oxidant used. These observations also suggest that the effects of oxidative stress on pHᵢ involve different mechanisms and that different cells likely have different targets of oxidative stress. Therefore, our results provide important insight into the effects of oxidative stress on the central nervous system (CNS). Whether oxidative stress has similar effects on neurons from different regions, including nonchemosensitive areas of the brain stem or cortical regions known to be sensitive to oxidative stress (e.g., substantia nigra), remains to be determined.

**Mechanism of oxidant-induced intracellular acidosis.** Our results show that oxidant-induced acidification of SC neurons occurred in conjunction with a decrease in amiloride-sensitive NHE activity. This observation is consistent with previous studies conducted in nonneuronal preparations that reported NHE activity is affected by oxidative stress. For example, H₂O₂, hyperoxia, and the chemical oxidant t-BHP decreased NHE activity and acidified arterial endothelial cells (12, 13, 29), atrial myocardial cells (9), and renal epithelial cells (33). Furthermore, in those preparations where oxidative stress has the opposite effect on pHᵢ, it also had the opposite effect on NHE activity; i.e., ROS produced by hyperoxia or ferric nitroliotriacetate increased NHE activity and alkalized kidney epithelial cells (17) and hepatic stellate cells (60). These results suggest that the effects of oxidative stress on NHE activity contribute to their effects on pHᵢ. However, Chambers-Kersh et al. (6) showed that inhibition of NHE under control conditions with amiloride did not acidify neurons in the nucleus tractus solitarius (i.e., dorsal SC). Therefore, other factors in addition to decreased NHE activity likely contribute to the oxidant-induced acidification observed in this study.

Possible factors in addition to NHE inhibition that may contribute to the acidification caused by oxidative stress include 1) metabolic acidosis (8); 2) ATP hydrolysis (63); 3) H⁺ redistribution (33, 54); 4) decreased pH buffer capacity (29, 33); or 5) with regard to CT- and NCS-induced acidification, the formation of HCl as a by-product of CT- or NCS-mediated protein oxidation. Regarding the first possibility, there is evidence that oxidative stress disrupts components of the electron transport chain (8), thereby slowing or halting oxidative phosphorylation and resulting in increased lactic acid production. In support of this possibility, oxidative stress has been shown to increase lactate production in the CNS (7), and hyperoxia has been shown to decrease metabolism in brain slices (36). The buffering power of cells in this region is high at 45 mM/pH unit (6). Therefore, to change pHᵢ by 0.20 pH units would require ~9 mM lactic acid (1 H⁺ per lactic acid) to be produced during oxidative stress. This value is similar to the transient increases in lactate levels reported to occur during focal brain activation, ~6 mM (24). Regarding the second possibility, H₂O₂ has been shown to increase ATP hydrolysis, which caused a pronounced decreased pHᵢ (63). However, for ATP breakdown to decrease pHᵢ by 0.20 pH units would require the hydrolysis of ~9 mM ATP (1 H⁺ per ATP). This value exceeds total intracellular ATP levels in the brain, which range from ~4.6 to 6.4 mM (4, 58). These first two possibilities would both lead to depleted levels of intracellular ATP, which itself can cause a third possibility, i.e., redistribution of H⁺ from acidified organelles (e.g., lysosomes) to the cytoplasm by inhibition of the H⁺-ATPase (33). It is also important to note that ATP depletion can also decrease sensitivity of NHE to intracellular H⁺ (16) and could be part of the mechanism by which oxidative stress slows NHE activity. Evaluation of the possible contribution of these mechanisms must await measurements of lactate, phosphocreatine, ATP, or other metabolic by-products during oxidative stress in SC neurons.

Regarding the fourth possibility, previous studies showed that concentrations of H₂O₂ that decreased pHᵢ and inhibited NHE activity did not affect pH buffer capacity (29, 33, 54). These results suggest that oxidative stress does not affect pHᵢ buffer capacity. Regarding the final possibility, although it is theoretically possible that CT and NCS produce acid (e.g., HCl) as a by-product of protein oxidation, we are unable to find any experimental evidence to support this possibility. These possibilities are not mutually exclusive but, rather, likely occur in concert such that the effect of oxidative stress on pHᵢ involves a combination of these effects.

**Effects of CT on neuronal excitability.** The SC contains CO₂/H⁺-chemosensitive neurons that respond to changes in CO₂ by a mechanism that involves decreased pHᵢ (53). Recently, it was shown that CO₂/H⁺ chemoreceptors are highly sensitive to hyperoxia by a mechanism that appears to involve ROS (38) and the redox signaling pathway (18). We have shown here that chemical oxidants and ROS decrease pHᵢ. Together, these results beg the question, does oxidative stress stimulate SC neurons directly by redox modulation of ion channels or indirectly by decreased pHᵢ? To test this possibility, we made WCR of Vᵢm while measuring pHᵢ in SC neurons during exposure to CT.

**Use of WCR technique to study oxidant sensitivity of SC neurons.** The main advantage of measuring Vᵢm by using the whole cell configuration over other patch-clamp recording methods is that pharmacological agents or fluorescent dyes in the pipette can be rapidly introduced into the cell (28). For
example, in this study, using the whole cell configuration, we were able to rapidly load SC neurons with the membrane-impermeable fluorescent dye pyranine to perform simultaneous measures of \( V_m \) and \( \mathrm{pH}_i \). However, this advantage also results in the major disadvantage of the WCR technique, that being washout. The rapid exchange between the pipette solution and cytoplasmic material results in dilution or washout of soluble intracellular molecules (28), including important components of the \( \mathrm{CO}_2/\mathrm{H}^+ \)-chemosensitive signaling mechanism (15, 22, 47). To some extent we were able to ameliorate the effects of washout on chemosensitivity by lowering EGTA-Ca\(^{2+}\) concentration in the whole cell filling solution as described previously (22). However, low-EGTA-Ca\(^{2+}\) whole cell solution did not prevent washout in all cells (e.g., Fig. 7). Therefore, block the effects of washout on \( \mathrm{CO}_2/\mathrm{H}^+ \) chemosensitivity in SC neurons.

**Oxidative stress stimulates SC neurons independently of changes of \( \mathrm{pH}_i \).** CT in the presence of either high \( \mathrm{HCO}_3^- \) or low \( \mathrm{CO}_2 \) stimulated firing rate in 83% of SC neurons. Furthermore, there was no difference in the firing rate response evoked by CT in either high \( \mathrm{HCO}_3^- \) or low \( \mathrm{CO}_2 \), indicating that SC neurons are responding to CT and not high \( \mathrm{HCO}_3^- \), as previously shown to occur in hippocampal neurons (5). Our finding that CT stimulates firing rate in the absence of a change in \( \mathrm{pH}_i \) suggests the possibility that oxidative stress stimulates SC neurons by a direct mechanism, possibly involving the redox modulation of cysteine and/or methionine on an as-yet unidentified K\(^+\) channel.

There is increasing evidence that redox modulation of ion channels is an important signaling mechanism for controlling neuronal excitability (18). Our results are consistent with previous studies that showed oxidative stress to decrease K\(^+\) channel conductance. For example, CT or ROS decreased conductance of delayed rectifier (19, 59) and A-type K\(^+\) channels (1) and increased or decreased Ca\(^{2+}\)-dependent K\(^+\) channel conductance, depending on whether a cysteine or methionine was oxidized (61). Together, these results support the possibility that transmembrane proteins such as NHE or K\(^+\) channels can be redox modulated in ways that affect neuronal activity.

**Contribution of \( \mathrm{pH}_i \) to the CT response.** Oxidative stress and \( \mathrm{CO}_2 \) both increase activity of SC neurons, by increased ROS production (38) and decreased \( \mathrm{pH}_i \) (21, 53), respectively. Our results show that the effects of CT or NCS and \( \mathrm{CO}_2 \) on \( \mathrm{pH}_i \) are additive. Furthermore, we recently showed that the firing rate response to \( \mathrm{CO}_2 \) plus hyperoxia was equal to or greater than the sum change in firing rate evoked by each stimulus alone (38). Therefore, we hypothesized that the CT-induced acidification would contribute to the CT response of SC neurons. Our results did show that CT increased firing rate somewhat more than \( \mathrm{CO}_2 \) in the presence of high \( \mathrm{HCO}_3^- \) or low \( \mathrm{CO}_2 \), but this difference was not significant. However, this result should be interpreted with caution because our sample size was relatively small. We anticipate that with a larger sample size the difference between CT and CT in high \( \mathrm{HCO}_3^- \) or low \( \mathrm{CO}_2 \) might become significant. Regardless, our results indicate that the CT-induced acidification is not required for the oxidant-induced firing rate response of SC neurons.

It is unclear why the CT-induced acidification does not appear to result in further significant stimulation of the firing rate of SC neurons. It is possible that factors in addition to decreased \( \mathrm{pH}_i \) are required for neuronal stimulation. For example, Filosa and Putnam (22) showed that propionate decreased \( \mathrm{pH}_i \) by ~0.2 pH unit but did not increase firing rate of locus coeruleus neurons. This possibility is further supported by a phenomenon known as the hypoxia paradox; i.e., hypoxia-induced intracellular acidification does not increase activity of \( \mathrm{CO}_2/\mathrm{H}^+ \) chemoreceptors to stimulate ventilation (41). These results suggest that decreased \( \mathrm{pH}_i \) by itself does not necessarily stimulate activity of \( \mathrm{CO}_2/\mathrm{H}^+ \) chemoreceptors. However, more experiments are required to confirm this possibility.

**Model of \( \mathrm{CO}_2/\mathrm{H}^+ \)- and oxidative stress-induced signaling pathways in SC neurons.** \( \mathrm{CO}_2 \) provides the primary stimulus for breathing, in part, by stimulating \( \mathrm{CO}_2/\mathrm{H}^+ \)-chemosensitive neurons located in various brain stem regions (53). \( \mathrm{CO}_2/\mathrm{H}^+ \)-chemosensitive neurons in the SC are also sensitive to various forms of oxidative stress, including hyperoxia and chemical oxidants (38). Figure 10 presents our current working model summarizing the effects of \( \mathrm{CO}_2 \) and oxidative stress on \( \mathrm{CO}_2/\mathrm{H}^+ \)-chemosensitive neurons. The effect of \( \mathrm{CO}_2 \) and oxidative stress on the excitability of SC neurons appears to be mediated...
by separate mechanisms; CO₂ involves changes in pH_i (21), and oxidative stress in the form of CT, NCS, or hyperoxia involves oxidation (38). We have shown here that oxidative stress can decrease pH_i and NHE activity; therefore, decreases in pH_i (Fig. 10, purple box) are an area of intersection between the two signaling mechanisms. The importance of the oxidant-induced acidification and the potential for an interaction between CO₂ and oxidative stress signaling mechanisms remain to be determined.

Our results suggest that oxidant-induced acidification is not required for the oxidant signaling mechanism. Chemosensitive neurons are sensitive to hypercapnic acidosis (30, 53). It is still conceivable, however, that the oxidant-induced acidification contributes to the effects of oxidative stress on SC neurons, if not through the CO₂/H⁺ signaling mechanism, then possibly by amplifying the oxidant signal. There are several ways in which intracellular acidosis might increase oxidative stress (Fig. 10, arrow 13). These possibilities include 1) acidification-induced dissociation of iron from transferrin (26) and possibly ferritin (44) to facilitate iron catalysis of O₂⁻ and H₂O₂ to the very reactive OH⁻ radical (26); 2) acidification-induced increases of the formation of reactive nitrogen species (32); 3) acidification-induced alteration of redox reactions, many of which are pH dependent (52); and 4) acidification-induced decreases in enzymatic antioxidant activity (66). These possibilities are supported by previous studies that have demonstrated that production and/or stability of ROS including O₂⁻, H₂O₂, OH radical, and/or peroxynitrite are increased at more acidic conditions (pH ranging from 7.0 to 6.1) (35, 45, 57, 66). In addition, decreasing bath pH from 7.2 to 6.2 decreased acidification caused by oxidant stress in the form of CT, NCS, or hyperoxia affects respiratory control is unknown. Hyperoxia has been shown to destabilize breathing in some infants presenting with recurrent apnea and cyanosis (3). Furthermore, chronic oxidative stress in the form of high iron or alterations in CNS antioxidant levels are thought to contribute to sudden infant death syndrome (31, 46). Our observations that oxidative stress decreases pH_i and NHE activity and increases excitability of neurons in a respiratory control brain stem region suggests a possible mechanism by which oxidative stress affects and possibly disrupts respiratory control.

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References
23. Gan BS, Krump E, Shrode LD, and Grinstein S. Loading pyranine via
puriﬁnergic receptors or hypotonic stress for measurement of cytosolic pH

24. Griffith JL, Rae C, Radda GK, and Matthews PM. Lactate-induced
inhibition of glucose oxidation in guinea pig cortical brain slices. Neu-

25. Grinstein S and Furuya W. Cytoplasmic pH regulation in phorbol
C65, 1986.

26. Halliwell B and Gutteridge JMC. Oxygen toxicity, oxygen radicals,

27. Hirpaara J, Clement MV, and Pervais S. Intracellular acidiﬁcation
triggered by mitochondrial-derived hydrogen peroxide is an effecter
mechanism for drug-induced apoptosis in tumor cells. J Biol Chem 276:

28. Horn R and Korn SJ. Prevention of rundown in electrophysiologically

29. Hu Q, Xia Y, Corda S, Zweier JL, and Ziegelstein RC. Hydrogen
peroxide decreases pH in human aortic endothelial cells by inhibiting

30. Huang RQ, Erlichman JS, and Dean JB. Cell-cell coupling between
CO2-excited neurons in the dorsal medulla oblongata. Neuroscience 80:

31. Huggle S, Hunsaker JCIII, Coyne CM, and Sparks DL. Oxidative

32. Huie RE and Neta P. Chemistry of oxygen species. In: Reactive Oxygen
Species in Biological Systems, edited by Gilbert D and Colton C. New

of cell pH regulation by H2O2 in renal epithelial cells. Arch Biochem Biophys

34. Loh SH, Tsai CS, Tsai Y, Chen WH, Hong GJ, Wei J, Cheng TH, and
Lin CI. Hydrogen peroxide-induced intracellular acidosis and electrome-
chanical inhibition in the diseased human ventricular myocardium. Eur

35. Miura I, Miyamoto K, Nakamura K, and Watanabe Y. Hydrogen
peroxide induced chemokine production in the glia-rich cultured cerebellar

36. Mulkey DK, Henderson RA III, Olson JE, Putnam RW, and Dean JB.
Oxygen measurements in brainstem slices exposed to normobaric hyper-

40. Rechnrona S, Hauge RN, and Siesjo BK. Enhancement of iron-cata-
yzed free radical formation by acidosis in brain homogenates: difference
in effect by lactic acid and CO2. J Cereb Blood Flow Metab 9: 65–70,
1989.

41. Reid GM and Tertit H. Sudden infant death syndrome: oxidative stress.

42. Richerson GB. Response to CO2 of neurons in the rostral ventral medulla

43. Ritucci NA, Chambers-Kersh L, Dean JB, and Putnam RW. Intracel-
luar pH regulation in neurons from chemosensitive and nonchemosensi-
tive areas of the medulla. Am J Physiol Regul Integr Comp Physiol 275:

44. Ritucci NA, Dean JB, and Putnam RW. Intracellular pH response to
hypercapnia in neurons from chemosensitive areas of the medulla. Am J

45. Ritucci NA, Erlichman JS, Dean JB, and Putnam RW. A ﬂuorescence
technique to measure intracellular pH of single neurons in brainstem

46. Ritucci NA, Putnam RW, and Dean JB. Simultaneous measurement of
intracellular pH and membrane potential in locus coeruleus neurons during

47. Schaefer FQ and Buettner GR. Redox environment of the cell as viewed
through the redox state of the glutathione disufide/glutathione couple.

48. Scheid P and Putnam RW, Dean JB, and Ballantyne D (Guest Editors).

49. Shaw S, Naegeli P, Etter JD, and Weidmann P. Inhibition of rat
glomerular mesangial cell sodium/hydrogen exchange by hydrogen per-

50. Sheltzer J, Burstyne Y, and Patchornik A. Selective oxidation of

51. Shiibunuma M, Kuroki T, and Nose K. Superoxide as a signal for

52. Shu Z, Jung M, Beger HG, Marzinzig M, Han F, Butzer U, Bruckner
UB, and Nussler AK. pH-dependent changes of nitric oxide, peroxyni-
trite, and reactive oxygen species in hepatocellular damage. Am J Physiol

53. Silver IA, Deas J, and Erecinska M. Ion homeostasis in brain cells:
differences in intracellular ion responses to energy limitation between

54. Stephens GJ, Owen DG, and Robertson B. Cysteine-modifying reagents
alter the gating of the rat cloned potassium channel Kv1.4. Pﬂugers Arch

55. Svegliati-Baroni G, Di Sario A, Casini A, Ferretti G, D’Ambrosio L,
Rizzoli F, Bolognini L, Salzano R, Orlandi F, and Benedetti A. The Na+/H+
exchange modulates the ﬁbrogenic effect of oxidative stress in rat hepatic

56. Tang XD, Daggett H, Hanner M, Garcia ML, McManus OB, Brot N,
Weissbach H, Heinemann SH, and Hoshi T. Oxidative regulation of
large conductance calcium-activated potassium channels. J Gen Physiol

57. Thomas JA, Buchsbaum RN, Zimmaka I, and Racker E. Intracellular
pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic

58. Tsai KL, Wang SM, Chen CC, Fong TH, and Wu ML. Mechanism of
oxidative stress-induced intracellular acidosis in rat cerebellar astrocytes

model of the human Na+/H+ exchanger isoform 1. J Biol Chem 275:

60. Wu ML, Tsai KL, Wang SM, Wu JC, Wang BS, and Lee YT. Mechanism of

61. Ying W, Han SK, Miller JW, and Swanson RA. Acidosis potentiates
oxidative neuronal death by multiple mechanisms. J Neurochem 73: