Key contributions of the Na\(^+\)/H\(^+\) exchanger subunit 1 and HCO\(_3\)\(^-\) transporters in regulating neuronal cell fate in prolonged hypoxia

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HCO\(_3\)\(^-\) transporters and the Na\(^+\)/H\(^+\) exchanger (NHE) contribute in a major way to maintenance of ionic and pH homeostasis in neurons. The study by Xue et al. (17) demonstrates that, in prolonged neuronal hypoxia, inhibition of HCO\(_3\)\(^-\) transporters by DIDS is protective and inhibition of NHE by either HOE 643 or T-162559 results in increased cell death. These observations have important implications for potential therapeutic interventions that could target upregulation of NHE activity in diseases marked by prolonged hypoxia. The protective role of NHE function in prolonged hypoxia contrasts markedly with a prodeath role for HCO\(_3\)\(^-\) transporters under conditions of prolonged hypoxic exposure.

THE NHE AND HCO\(_3\)\(^-\) TRANSPORTERS IN BRAIN

The ionic and pH homeostasis in neurons and glia is regulated to a major degree by two families of acid-base transporters, the NHE family and the HCO\(_3\)\(^-\)-dependent acid-base transport protein family. The latter family includes the Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\)\(^-\) anion exchanger (AE1-4), the Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\)\(^-\) exchanger, and a number of Na\(^+\)-HCO\(_3\)\(^-\) cotransporters (NBC). The NHE subunit 1 (NHE1), NBC, AE3, and NBC are key contributors to acid-base regulation in neurons and glia. As emphasized in the study by Xue et al. (17) on cultured neurons subjected to long-term hypoxia, ionic disturbances involving modulated activity of the NHE and of members of the HCO\(_3\)\(^-\) transporter family may contribute in important ways to regulating levels of neuronal hypoxic injury. A key observation in this study is that the NHE plays a prosurvival role in neurons under conditions of prolonged hypoxia. The protective action of the NHE in hypoxic neurons is in direct contrast to its reported function in ischemic heart where NHE blockers are proposed for therapeutic interventions. In this investigation, pharmacological inhibition of HCO\(_3\)\(^-\) transporters using the reagent 4,4′-disulfonate (DIDS) prevented hypoxic cell death in cultured neurons and hippocampal slices.

Depending on the model system, pharmacological blocking of the NHE may be either prosurvival or death inducing. For example, in extensive investigations in models of myocardial ischemia, it has been reported that NHE1 inhibition in ischemia with reperfusion results in potent myocardial protection (2). This prosurvival effect of NHE1 inhibition appears to be mediated by a decrease in intracellular Na\(^+\) that consequently prevents intracellular Ca\(^{2+}\) overload arising via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange. In a two-vessel occlusion model of cerebral ischemia-reperfusion injury, 5-(N-ethyl-N-isopropyl)amiloride, an Na\(^+\)/H\(^+\) exchange inhibitor, resulted in decreased CA1 pyramidal neuron loss (15). Although the previously mentioned studies point to NHE inhibition as protective, a common theme in such investigations is the necessity for reperfusion to reveal such a role for NHE inhibitors. Critically, in a number of cell types, including renal tubular epithelial cells and cerebellar granule neurons, inhibition of NHE contributes in an important way to activation of specific cell death pathways.
HCO$_3^-$ transporters may be classified as either Na$^+$-coupled HCO$_3^-$ transporters or AE anion exchangers, which are Cl$^-$/HCO$_3^-$ exchangers. It has been demonstrated previously that a DIDS-sensitive Na$^+$- and HCO$_3^-$-dependent mechanism, possibly a Na$^+$/HCO$_3^-$ cotransporter, in addition to a NHE act to maintain steady-state pH$_i$ in cultured mouse cortical neurons. pH$_i$ recovery after acid loading is dependent primarily on a NHE and a DIDS-sensitive Na$^+$-dependent Cl$^-$/HCO$_3^-$ exchanger. In the study by Xue et al. (17), DIDS, which is capable of inhibiting HCO$_3^-$ transporters and Cl$^-$ channels, was protective against neuronal injury in cortical neuronal cultures and in hippocampal slices. The results from this investigation point strongly toward a role for the DIDS inhibition of a HCO$_3^-$ transporter in addition to the inhibition of the NHE by either HOE 643 or T-162559 in pH$_i$ regulation in prolonged hypoxia. Because of the protective nature of the DIDS-induced inhibition of HCO$_3^-$ transporters in prolonged neuronal hypoxia, the DIDS-sensitive mechanism is proposed by the authors as a potential therapeutic target.

The role of HCO$_3^-$ transporters in brain has been investigated recently in vivo using a mouse model of prolonged hypoxia (4). In mice subjected to continuous chronic hypoxic over 2–4 wk, expression levels in brain for two electroneutral Na$^+$/HCO$_3^-$ transporters NBCn1 and NCBE were found to be decreased significantly. These HCO$_3^-$ transporters act as acid extruders and thus elicit a net uptake of HCO$_3^-$ and raise pH$_i$. The depletion in NBCn1 and NCBE in hypoxia would be likely to result in lower steady-state pH$_i$. This downregulation of NBCn1 and NCBE expression may be an energy-saving strategy for the cell, with the decreased pH$_i$ representing a biproduct of this strategy. A major theory of hypoxic adaptations derives from evidence that cells respond to oxygen deprivation by downregulating ATP generation as well as ATP-consuming processes (8, 9). Thus, for example, in hypoxia-tolerant cells, “channel arrest” involves a downregulation of ion pumping mechanisms that comprise a major component of cellular ATP utilization. In responding to chronic hypoxic stress, there is evidence for a global cellular strategy involving a generalized decrease in protein synthesis combined with preferential gene expression and selective translation of only specific messages (8). Ma and Haddad (13) have proposed that there is a hierarchy of proteins that may selectively be maintained or decreased in hypoxia, depending on cellular requirements. In the study by Chen et al. (4), the reported depletion in levels of electroneutral HCO$_3^-$ transporters in chronic hypoxia was out of proportion to a generalized decrease in protein levels and may therefore represent part of a selective and critical regulatory step in maintaining ionic and pH homeostasis in the brain.

**IMPORTANT DISTINCTIONS BETWEEN CHRONIC CONTINUOUS HYPOXIA AND CHRONIC INTERMITTENT HYPOXIA AS MODEL SYSTEMS**

Chronic continuous hypoxia (CCH) is distinguished from chronic intermittent hypoxia (CIH) by virtue of the latter’s clear use of ROS in the signaling processes associated with the resulting cellular adaptations. Thus CCH has been seen as a factor in certain physiological adaptations, including embryonic development and tissue alterations associated with living at high altitudes as well as in a variety of disease conditions, including anemia and ischemia. CCH results in a near doubling of capillary density in the brain, a process that is regulated by HIF-1 and angiopoietin-2 (10). By contrast, CIH may be associated with such diseases as obstructive sleep apnea/hypopnea (or hypoventilation) syndrome, and, in rats, CIH is related to a reversible defect in memory and motor function. A recent study related to brain acidemia in a mouse model of CIH has demonstrated decreased expression of NHE subunits and of AE3 anion exchanger and of NBC, especially in cerebellum and hippocampus (5). It was proposed that this decrease in acid-extruding proteins would contribute to the susceptibility of neurons to damage from acidosis and that these detrimental effects were, in part, linked to signaling that involves the increased ROS levels that are characteristic of the CIH model.

**IS THERE A ROLE FOR ROS SIGNALING IN PROLONGED HYPOXIA?**

Although ROS signaling is not regarded as central to CCH, it may be that a role for ROS signaling in prolonged hypoxia has not been precluded and, for example, HIF-1 activation in hypoxia is regulated by redox mechanisms in the dopaminergic PC-12 cell line (1). In examination of potential hypoxia-sensing mechanisms using a cardiac myocyte model, Chandel and Shumacker (3) have proposed a key sensing role for elevated cellular ROS based on initial studies on hypoxic cardiac myocytes (3, 6). This elevated cellular ROS is postulated to occur in hypoxia as a result of a decreased reduction of O$_2$ to H$_2$O by cytochrome oxidase, resulting in a release of electrons upstream at complex III of the mitochondrial electron transport chain and consequently generation of superoxide (6). Although this cardiac myocyte study was based on 1–2 h of hypoxia (a relatively short duration of hypoxia), a number of related investigations illustrate the potential for a key contribution of ROS generation in prolonged hypoxia. For example, in kidney-derived HEK293 cells subjected to prolonged hypoxia, mitochondrial ROS generation induced a HIF-1-independent depletion in cellular glutathione levels (14). In heart and skeletal muscle, ROS generation in chronic hypoxia may perform a protective function through modulation of Ca$^{2+}$ signaling, leading to an increased mitochondrial bioenergetic capacity (7).

**Under hypoxic conditions, does acidosis promote cell death or cell survival?** In the mouse model of continuous chronic hypoxia, the reported decrease in expression of HCO$_3^-$ transporters may represent an energy-saving strategy, and the result may be a reduced pH$_i$ and a decreased capacity of cells to respond to changes in intracellular acidity (4). In neuronal cultures subjected to prolonged hypoxia, acidosis would again be damaging. Under hypoxic conditions, pharmacological inhibition of the acid extruder NHE1 would increase intracellular acidification and in so doing contribute to neuronal damage and, potentially, enhancement of apoptotic processes (17).

The nature of the contributions of acidosis to neuronal cell death in hypoxic conditions is likely to depend on in part on the degree of the acidosis, with mild acidosis in some instances being seen as protective. In a model of focal brain ischemia, acidosis induced by hypercarbic ventilation resulted in decreased infarct volume relative to nonacidotic treatment, implicating moderate acidosis (optimally at brain pH 6.8) as protective against ischemic damage (11). In several in vitro
neuronal models, reduced pH is protective against ischemia and against glutamate. Reduced pH decreases N-methyl-D-glucamine (NMDA)-mediated Ca\(^{2+}\) entry, resulting in hypoxic neuronal protection in vitro (11, 18).

In common with the effects mentioned previously on ischemic heart, augmented intracellular acidification in ischemic brain could activate NHE function, causing a damaging increase in intracellular Na\(^{+}\) (11). Free radical formation may also be a potential mechanism for ischemic damage augmented by lowered pH, with such an effect occurring via iron delocalization and the Fenton reaction. In a model of focal brain ischemia, tissue damage was augmented by preexisting hyperglycemia with a greater than twofold elevation in free radical production (16, 11). In contrast to the NMDA receptor actions on in vitro neuronal cultures mentioned above, cell death mediated by the AMPA/kainate receptor contribution to ischemic cell death in neuronal cultures is augmented by acidosis. Lowered pH\(_6\) was reported to increase AMPA-kainate receptor-induced neurotoxicity in cultured neocortical cells (11).

SUMMARY/CONCLUDING REMARKS

In summary, the article by Xue et al. (17) is important in terms of demonstrating a protective effect on hypoxic neurons of DIDS-mediated inhibition of HCO\(_3^-\) transporters and an injurious effect of inhibiting NHE1 by either HOE 643 or 5-(N-ethyl-N-methyl-amiloride, an Na\(^+\)/H\(^+\) exchange inhibitor, protects gerbil ischemic rats during middle cerebral artery occlusion/reperfusion. Free Radic Biol Med 23: 986–995, 1997.


