The Journal of Experimental Biology 207, 3243-3249
Published by The Company of Biologists 2004
doi:10.1242/jeb.00977

Review

Clinical perspectives: neuroprotection lessons from hypoxia-tolerant organisms

Philip E. Bickler

Department of Anesthesia, University of California, San Francisco, CA 94143-0542, USA

e-mail: bicklerp@anesthesia.ucsf.edu

Accepted 12 March 2004

Summary

An effective treatment for brain ischemia is a pressing medical need. Research on brain ischemia has largely focused on understanding the mechanisms of neuron death as a way of identifying targets for therapy. An attractive alternative approach is to identify the survival strategies of hypoxia-tolerant neurons. The adaptation of vertebrate neurons to hypoxia occurs in at least three major ways: (1) as a constitutive property of neurons in anoxia-tolerant turtles and fish, (2) as a property of intra-uterine and early post-natal mammalian development, and (3) as part of a slower, chronic process, as in acclimitization to high altitude. Research on hypoxia-tolerant neurons has already revised several earlier concepts, including the role of calcium in cell death and survival, and the value of *N*-methyl-D-aspartate (NMDA) receptor antagonism. A broad and fundamental understanding of how neurons adapt to hypoxia is likely to help guide efforts to find new treatments for brain hypoxia and ischemia.

Key words: brain, ischemia, hypoxia, neuron, adaptation, hypothermia.

Introduction

Clinical neuroprotection: the challenge

Despite years of research, the treatment and prevention of hypoxic/ischemic brain injury remains a major medical challenge. The failure of numerous clinical trials seeking chemical neuroprotection for acute, ischemic stroke has been nothing short of humbling. Listed at the American Heart Association stroke website (http://www.strokecenter.org/trials), over 140 clinical trials of ischemic stroke treatments have been completed. Almost 250 drugs and combinations of drugs for ischemic and hemorrhagic strokes have been studied. Hundreds of millions of dollars invested in these pursuits are presently unrewarded with any breakthrough. Only revascularization of occluded cerebral arteries (e.g. the anti-clot treatments of embolectomy or thrombolysis) has been proven effective, and these can only work very early after a stroke, and not then for every patient.

The vast majority of the current research directed to finding treatments for ischemic or hypoxic brain injuries have been based on identifying, in molecular detail, what goes wrong when the brain is deprived of oxygen and nutrients. These efforts have identified hundreds of events in a number of complex and interactive cascades, which involve, very broadly, the following processes: (1) failure of energy balance (loss of ATP), (2) excitotoxicity (runaway excitatory neurotransmitter release), (3) free-radical damage, (4) inflammation and immune system over-activation, and (5) delayed cell death. Identifying the best target for therapy is thus not easy.

Developing a broad phylogenetic and evolutionary approach to treating brain hypoxia

The hypoxic/ischemic brain presents a myriad of possible targets for intervention. In addition, neurons are capable of mounting an elaborate series of defenses against hypoxic injury. A fundamental question is thus raised: when studying brain hypoxia or ischemia, how can damaging events be separated from useful defense mechanisms? In hypoxia-sensitive neurons the answer to this question is often not obvious. Hypoxia-tolerant neurons can provide critical insights into this problem. One may reasonably argue that, because studying how cells die has not yielded a treatment for hypoxic brain damage, it is time to study cells that survive oxygen lack. A main theme of this paper is that understanding the adaptive responses of neurons to hypoxia can be a very valuable tool for the pursuit of new neuroprotective strategies. Pursuing this theme, however, leads to a second and equally important question: how can such information be translated into new therapies? While it may be foolish to think that concepts can be directly transferred from the comparative physiology laboratory to the clinic, why shouldn't breakthroughs in our understanding of how cells adapt to hypoxic conditions eventually lead to treatments? Models of such transfer of information to clinical medicine include the alphastat concept of acid–base balance during cardiopulmonary bypass (derived from ectothermic vertebrates) and the use of hypothermia as a treatment for

heart attack and trauma (based on hibernation and winter dormancy).

Adaptive capacities of neurons to hypoxia: fertile ground for clinical lessons

All neurons have some capacity to adapt to changing oxygen tension. There are at least three fundamental, distinct and clinically relevant types of adaptation to hypoxia to consider: (1) *immediate adaptation*, the capacity of neurons from several types of extremely hypoxia-tolerant organisms (e.g. freshwater turtles, crucian carp) to be pre- or constitutively adapted to tolerate severe hypoxia; (2) *developmental adaptation*, the change in the functioning and tolerance of the CNS during ontogenetic changes in oxygen availability; and (3) *slow adaptation*, which is the capacity of neurons to gradually adapt to chronic, not acutely lethal, hypoxia. These are highlighted in Fig. 1. The capacity of neurons to adapt in each of these three ways can be truly remarkable. Undoubtedly, each adaptational theme will be best explored with a different set of models and experimental approaches. I believe that each has particular lessons of relevance for clinical consideration.

Immediate adaptation to anoxia

Mature neurons from some vertebrates appear immediately prepared to tolerate prolonged hypoxia, apparently without requiring gene expression or other preparatory adaptational adjustments. Probably the best-characterized examples of this type of neuron are those from the cerebrocortex of the freshwater turtle *Chrysemys picta*. Turtle neurons survive anoxia by means of a complex group of adaptations that include alterations in energy production, ion channel properties, neurotransmitter systems, neuromodulator levels, anti-apoptotic processes, and probably regeneration of lost neurons (reviewed by Lutz et al., 2003; Bickler and Donohoe, 2002; see also Lutz and Milton, 2004).

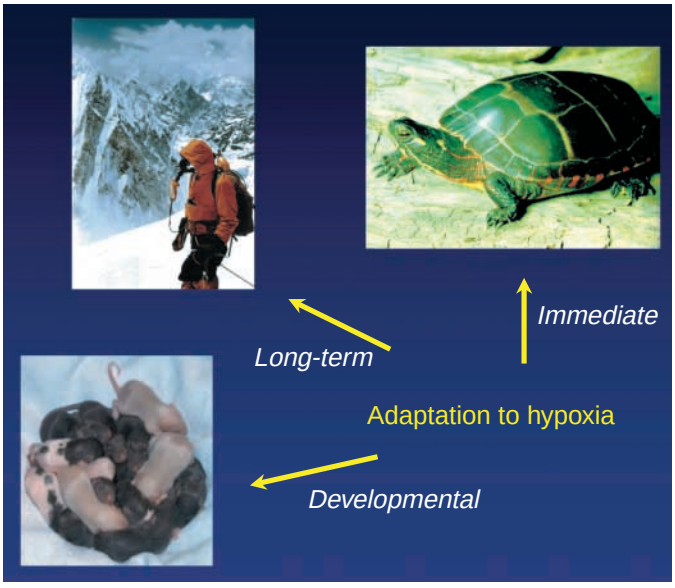

Fig. 1. Main themes of temporal patterns of hypoxia adaptation. (1) Immediate or constitutive, exemplified by the northern painted turtle, *Chrysemys picta*. (2) Developmental, as in the relative hypoxia of the *in utero* environment. (3) Slow or chronic, as exemplified by human adaptation to high-altitude hypoxia over the course of many days (photo courtesy of R. Boutilier).

There are several examples of how turtle neurons have provided new ideas or insights for neuroprotection concepts that may be useful clinically. One example concerns the role of Ca^{2+} in the death of hypoxic or ischemic neurons. Increases in intracellular calcium ($[Ca^{2+}]_i$) are considered central to ischemic injury in mammalian neurons, and have provided a target for numerous stroke therapy trials. Strikingly, moderate increases in $[Ca^{2+}]_i$ (50–300 nmol l^{-1}) are associated with long-term hypoxic survival of hypoxia-tolerant neurons from freshwater turtles (Bickler, 1998), *Rana* tadpoles (M. S. Hedrick and P. E. Bickler, personal observation), garden snails (P. H. Donohoe and P. E. Bickler, personal observation), and even hippocampal neurons from mammalian neonates (Bickler and Hansen, 1998). The proposition that moderate increases in calcium can be protective has some foundation in mammalian cells as well. Neuroprotective effects of moderate increases in $[Ca^{2+}]_i$ produced by depolarization or *N*-methyl-D-aspartate (NMDA) receptor activation have been observed in cultured mammalian neurons (Franklin and Johnson, 1994). Moderate increases in Ca^{2+} are essential for the activation and full expression of several critical survival pathways, including the MAPK p42/44 pathway (Fahlman et al., 2002) and the Akt pathways (Cheng et al., 2003). These pathways are best known for their neuroprotective effects mediated by activated growth factor receptors. In addition, hypoxia-induced changes in gene expression mediated by hypoxia inducible factor (HIF-1α) are modulated by Ca^{2+} *via* calmodulin and MAPKs (Mottet et al., 2003). A $[Ca^{2+}]_i$ of 50–100 nmol l^{-1} is typical of healthy neurons and an increase in $[Ca^{2+}]_i$ of 100–200 nmol l^{-1} is a signal associated with growth and development (Berridge et al., 2000). Conversely, excessively low $[Ca^{2+}]_i$ can promote apoptotic neuron death (Lampe et al., 1995). It is thus reasonable to conjecture that moderate $[Ca^{2+}]_i$ increases before, during, or after brain ischemia may be substantially more conducive to neuronal survival than low $[Ca^{2+}]_i$ during the same periods (Lee et al., 1999). This controversial hypothesis predicts that preventing all but large increases in $[Ca^{2+}]_i$ in neurons during or following brain ischemia may be detrimental.

Transferring concepts from turtles to mammalian neurons: the role of calcium

Because increases in calcium are associated with surviving anoxia in hypoxia-tolerant neurons, we have re-examined the role of calcium in cell death in mammalian neurons, testing the hypothesis that moderate increases in calcium are protective. We used the rat hippocampal slice culture (HSC) model (Stoppini et al., 1991) to explore this idea because it is

particularly suited to pharmacologic manipulations and the examination of delayed cell death. We find that by simply treating the HSCs with ionophores to increase $[Ca^{2+}]_i$ produces impressive resistance to subsequent oxygen and glucose deprivation. The mechanisms of this protective effect involve the activation of several recognized neuroprotective signaling cascades (MAPK ERK1/2 and Akt or protein kinase B). Blocking ERK or Akt or applying ionophores in calcium-depleted medium reverses the usual protection (Bickler and Fahlman, 2004). Further, these studies have has led us to appreciate that other pre-conditioning strategies may involve increases in $[Ca^{2+}]_i$ as a centrally important event. It is probable that Ca^{2+} is directly required as a potentiating cofactor for activation of critical signaling cascades. These are illustrated in Fig. 2. Transferred to the clinic, this concept has the potential of avoiding the deleterious effects of over-zealous calcium blockade. Of note, the list of failed stroke treatments involving blocking calcium increases is particularly long. Could this situation have been at least partially avoided by a more comprehensive understanding of calcium homeostasis in hypoxic neurons?

Other findings from turtle neurons are also relevant to clinical considerations. Studies of anoxia-tolerant neurons from freshwater turtle (*C. scripta*) cerebrocortex show that NMDA receptor function is decreased, but definitely not eliminated, during anoxia (Bickler, 1998; Bickler et al., 2000), and that glutamate release and re-uptake are modulated during anoxia (Milton et al., 2002). This again is a challenge to a long-held belief that NMDA receptors should be blocked to stop stroke damage. Finally, turtle neurons can inform us about the significance of complex signaling events during or following anoxia. The pro-apoptotic cofactor Bax is upregulated during re-oxygenation in anoxic turtle cerebrocortex (J. J. Haddad, unpublished data), possibly indicating that Bax is not always associated with death signaling, or at least that its role is more complex than currently appreciated.

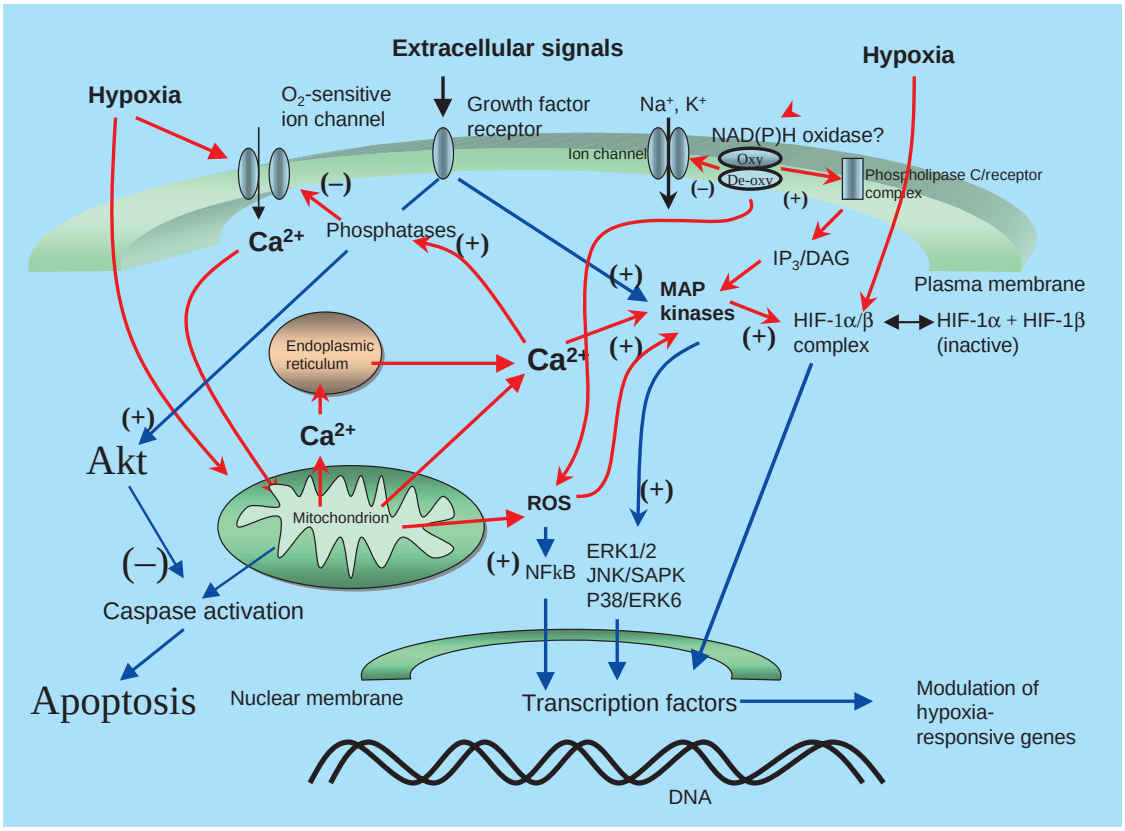

Fig. 2. Interaction of calcium in neuroprotection and oxygen-sensing mechanisms in vertebrate neurons. Rapid responses to hypoxia are shown in red and more slowly developing responses are shown in blue. (+) indicates a potentiating effect on the target, (–) indicates an inhibitory one. Oxygen interacts with a variety of target molecules, both at the cell surface, e.g. NMDA receptors (Bickler et al., 2003), K^+ channels and NADPH oxidase (Prabhakar and Overholt, 2000), and in the cytosol (e.g. HIF-1 and related proteins; Semenza, 1999) in processes that require Ca^{2+} (Mottet et al., 2003). Hypoxia has indirect effects mediated by changes in the bioenergetic state of mitochondria that involve Ca^{2+} (Berridge et al., 2000; Bickler et al., 2000) and reactive oxygen species (ROS) (Haddad and Land, 2000). Signaling *via* the tyrosine-kinase receptor family also requires Ca^{2+} and results in activation of Akt, an inhibitor of apoptosis (Cheng et al., 2003). Growth factors (Nicole et al., 2001), cytokines and inorganic ions (Millhorn et al., 2000) also may modulate neuronal responses to hypoxia and depend on appropriate $[Ca^{2+}]_i$ for their action. Many of these signals converge on calcium-dependent MAP kinase cassettes including the ERK, JNK and p38 pathways (Mattson, 1997; Minet et al., 2000; Semenza, 1999). This figure was modified from Bickler and Donohoe (2002).

Developmental adaptation to hypoxia

Neonatal mammals

Studies of developmental changes in the adaptation of mammalian tissues to hypoxia should have a high probability of providing clinically relevant insights. After all, the phylogenetic barrier is much lower here.

It has been appreciated for centuries that the neonate is more tolerant of hypoxia than the adult (Boyle, 1725). Low *in utero* oxygen tension correlates with the significantly greater hypoxia tolerance of embryonic or neonatal rats and their neurons (Bickler and Hansen, 1998; Friedman and Haddad, 1993). One notable feature of neonatal neurons is that they have the capacity to avoid excessive calcium influx mediated by glutamate receptors and other mechanisms (Bickler and Hansen, 1998; Friedman and Haddad, 1993). During hypoxia, neonatal neurons avoid death even though the concentration of glutamate in the brain tissues may be sufficient to saturate glutamate receptors (Bickler and Hansen, 1998; Cherici et al., 1991; Marks et al., 1996; Puka-Sundvall et al., 1997). Glutamate excitotoxicity is a significant threat to immature neurons when oxygen is present (Ikonomidou et al., 1989). It is therefore striking that the NMDA receptors that predominate in the neonatal brain generate large calcium currents (Burgard and Hablitz, 1993; Hestrin, 1992), which, although critical for strengthening of synapses (Durand et al., 1996; Tovar and Westbrook, 1999), would seem to increase the severity of glutamate excitotoxicity during hypoxic stress.

The observation that hypoxia silences NMDARs in turtle neurons has produced interesting new perspectives on the role of hypoxia during development and the innate hypoxia-tolerance of neonatal tissues. We hypothesized that hypoxia inhibits NMDA currents in neurons from the mammalian neonate just as it does in turtles. This proposition must take into account developmental changes in oxygen tension (*in utero* brain tissue oxygen tension is about 10 mmHg and increases to 30 mmHg at birth) as well as the significant changes in NMDAR composition and properties during the perinatal period. During the first 2–3 weeks of life, the NMDA receptor subunits NR2A and NR2C are added to or replace NR2B and NR2D subunits in functional receptors (Dunah et al., 1996; Wang et al., 1995; Wenzel et al., 1996). The increase in NR2A and NR2C subunits in cortex, hippocampus and cerebellum over the first few weeks of life almost exactly parallels the decrease in the hypoxia-tolerance of neonatal rats (Adolph, 1948) and their neurons (Bickler and Hansen, 1998; Friedman and Haddad, 1993).

It was significant, therefore, that we found that NR1/NR2D receptor currents were decreased by hypoxia and that NR1/NR2C receptor currents were increased by hypoxia (Fig. 3; Bickler et al., 2003). These studies were done using a *Xenopus* oocyte expression system, because NR2D expression in hippocampus peaks between birth and 1 week of age (Dunah et al., 1996; Kirsen et al., 1999; Wenzel et al., 1996). This effect may explain the inhibition of NMDAR responses during hypoxia in immature hippocampal neurons. In contrast, hypoxia activated receptors containing the NR2C subunit. Whereas NR2C subunits are scarce in the neonatal brain, they are expressed in the cerebellum and other areas after several weeks of postnatal life (Zhong et al., 1995). The pattern of appearance of NR2C receptors in the cerebellum fits the behavioral effects of hypoxia in rats: in neonates, hypoxia rapidly induces motor retardation, but in animals older than 2 weeks hypoxia at least transiently causes increases in motor activity. The hypoxia-tolerance of rat pups is greatly reduced after 2 weeks of life (Duffy et al., 1975).

An additional and novel insight from this work concerns the increase in oxygen tension at birth; oxygen is probably a signal for synaptic development because it promotes greater currents through NMDARs. The transition from the *in utero* level of ca. 10 mmHg to 30 mmHg is sufficient to do this. It is of interest that while normal oxygen levels are predicted to promote synapse formation, hypoxia would have the opposite effect and possibly retard normal synaptic development at a crucial period.

There are many fundamental unanswered questions concerning the neonate. One is how the neonatal brain avoids ATP loss during hypoxia. This response may be absolutely fundamental, and shared by all hypoxia tolerant cells (Hochachka, 1986; Hochachka et al., 1996). Are neonatal mitochondria more efficient producers of ATP? Neonatal neurons appear to ‘shut-down’ and adopt a stasis-like hypometabolic condition that is common to other anoxia-tolerant organisms (Hochachka and Guppy, 1987). Understanding this posture could pinpoint new approaches to helping vulnerable cells adapt to hypoxic conditions.

Slow adaptation

Chronic hypoxia and low brain tissue P_{O_2}

Adult mammalian neurons can adapt to very low oxygen tensions, given time. A striking example is the adaptation of the human CNS to high-altitude hypoxia. Very little is known mechanistically about how this occurs, or its limits and long-term effects. Tissue hypoxia at high altitude can be very impressive. For example, on the recent British Medical Research Expedition to Makalu in the Himalayas, one member of the scientific team had an arterial oxygen saturation of 43% after a 17-day approach hike (Gerald Dubowitz, personal communication). Assuming a significant respiratory alkalosis (pH_a 7.55) and increased 2–3-DPG, arterial P_{O_2} can be estimated at about 23 mmHg (normal at sea level would be 95 mmHg). This means that brain tissue P_{O_2} must be <10 mmHg! Remarkably, this individual’s mental status was normal. This degree of arterial or tissue hypoxia, if imposed acutely, would be lethal. At present one can only speculate that the adaptation of mammalian neurons follows some of the same principles already defined for more hypoxia-tolerant neurons, including increased reliance on glycolytic energy metabolism, increased mitochondrial efficiency (heresy to speak of this in a mammalian system), decreased sodium and

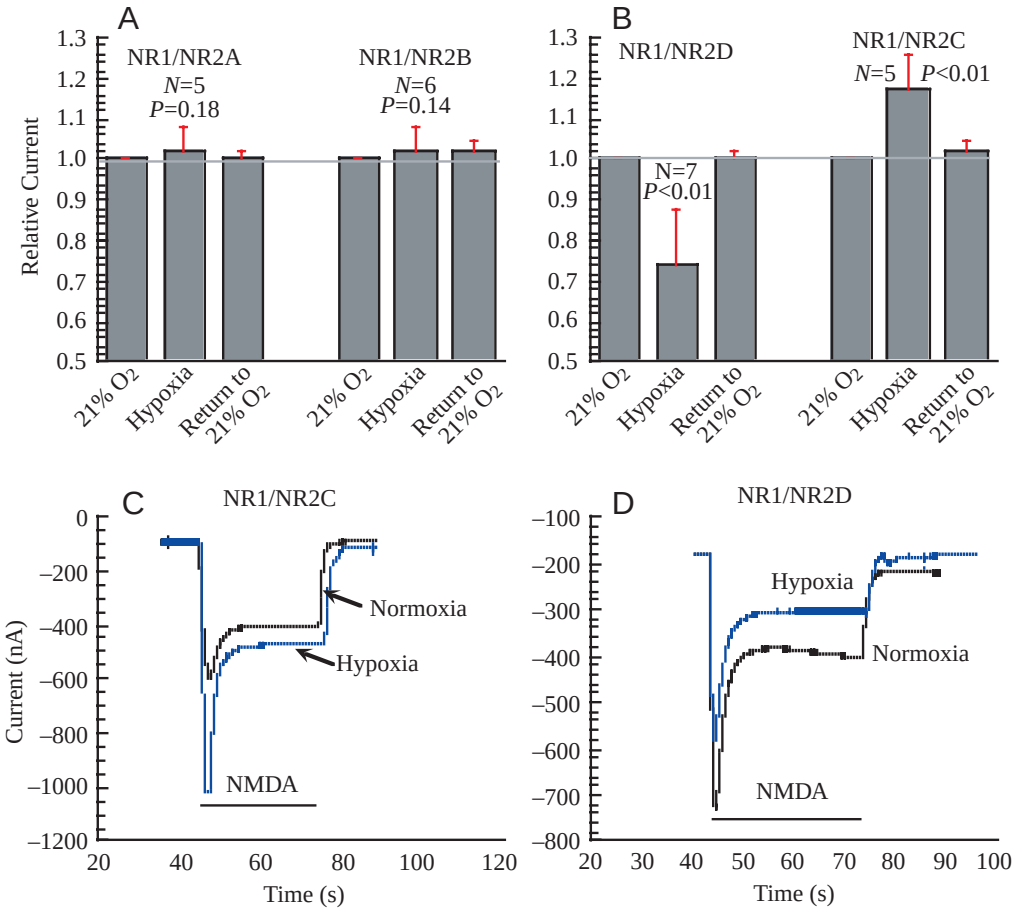

Fig. 3. Effects of oxygen on recombinant NMDA receptor currents in *Xenopus* oocytes. Hypoxia activates or inhibits cloned NMDARs expressed in *Xenopus* oocytes depending on the NR2 subunit. (A) Hypoxia has no effect on currents from NR1/NR2A and NR1/NR2B receptors. (B) Hypoxia inhibits currents from NR1/NR2D and augments those from NR1/NR2C. (C) Example of the augmenting effects of hypoxia on currents recorded from an oocyte expressing NR1/NR2C receptors. (D) Example of the inhibition of currents from NR1/NR2D during hypoxia. Reprinted from Bickler et al. (2003), with permission.

water transport, increased free-radical defenses (which seem to be very important in turtles; Rice et al., 1995), modulation of the calcium/cell death set-point, etc. These explorations are fertile areas for real advances – no question of clinical relevance here. Speeding up the process of adaptation to chronic hypoxia could be a valuable approach in treating diseases associated with hypoxia such as respiratory failure from pneumonia.

In contrast, intermittent hypoxia destroys neurons and provides a valuable contrast for adaptation to chronic hypoxia. For example, rats tolerate intermittent hypoxia very poorly, with even mild hypoxia (10% oxygen) coming in 90 s episodes during the night, causing death of hippocampal neurons and induction of a number of ‘stress genes’ (Gozal et al., 2002). Clinically, individuals with the intermittent hypoxia of sleep apnea suffer memory loss. Experimental models of chronic *versus* acute hypoxia could help identify injurious *versus* adaptive events.

Risks of engineering hypoxia tolerance: oncogenesis?

Oxygen is also a signal important to oncogenesis, to the growth of tumors and the growth of blood vessels within them. There is now preliminary evidence that hypoxia-tolerance mechanisms may confer survival advantage to tumors, or may themselves be oncogenic. For example, KCNK9 (TASK 3) potassium channels are upregulated in breast cancer, and when these channels are transfected into other cells they make the cells hypoxia tolerant, probably because they hyperpolarize the cell during impending energy failure (Pei et al., 2003). We have also demonstrated this tolerance in cultured hippocampal slices transfected using a Sindbis virus vector (C. S. Yost and P. E. Bickler, unpublished data). Not all potassium channels are protective, for unclear reasons. The enthusiasm for potassium channels as a strategy for stroke treatment may be dampened by these considerations.

Another case of pre-adaptation: hypothermia-associated hypoxia tolerance

Hypothermia may be one of our most valuable clinical tools in the search for treatments for brain ischemia. All known hypoxia-tolerant neurons tolerate and benefit substantially from hypothermia. Euthermic mammalian tissues do not

tolerate hypothermia for prolonged periods; problems include cellular edema, energy loss, activation of stress responses and disorders of intermediary metabolism, blood coagulation, autonomic regulation, electrical excitability and stability of the heart, to name a few. Additional clinical issues include altered drug clearance and increases in metabolism during rewarming (shivering can lead to myocardial ischemia in some individuals – a stress test). Increasing the hypothermia tolerance of tissues could possibly allow hypoxia tolerance to be achieved at the same time (Hochachka, 1986).

Conclusions and clinical lessons

Comparative physiology has already contributed in a significant way to medical science. Examples include alphastat regulation of blood pH for hypothermic surgery and hypothermia for traumatic injuries and heart attacks. Furthermore, I believe that this review has demonstrated that hypoxia tolerant neurons are a potential source of new strategies in our search for brain protection. Studies on these cells also remind us that we are at a very early stage in our understanding of how cells and tissues develop resistance to hypoxia. Hypoxia tolerant cells are very valuable models for understanding oxygen signaling processes simply because the responses to hypoxia are well developed. The possibility of separating adaptive signaling or defense responses from injury is a major benefit of studying hypoxia tolerant cells. They also serve as models for the slow adaptation of tissues to hypoxia, which humans are clearly capable of, and which might be enhanced to improve adaptation to diseases involving oxygen deficits.

This essay was written in memory of Peter Hochachka, whose work inspired many of the ideas presented. Supported by NIH grant RO1 NIGMS 52212-7.

References

Adolph, E. (1948). Tolerance to cold and anoxia in infant rats. *Am. J. Physiol.* **155**, 366-377.

Berridge, M., Lipp, P. and Bootman, M. (2000). The versatility and universality of calcium signaling. *Nat. Rev. Mol. Cell Biol.* **1**, 11-21.

Bickler, P. E. (1998). Reduction of NMDA receptor activity in cerebrocortex of turtles (*Chrysemys picta*) during 6 wk of anoxia. *Am. J. Physiol.* **275**, R86-R91.

Bickler, P. E. and Donohoe, P. H. (2002). Adaptive responses of vertebrate neurons to hypoxia. *J. Exp. Biol.* **205**, 3579-3586.

Bickler, P. E., Donohoe, P. H. and Buck, L. T. (2000). Hypoxia-induced silencing of NMDA receptors in turtle neurons. *J. Neurosci.* **20**, 3522-3528.

Bickler, P. E. and Fahlman, C. S. (2004). Moderate increases in intracellular calcium activate neuroprotective signals in hippocampal neurons. *Neurosci.* (in press).

Bickler, P. E., Fahlman, C. S. and Taylor, D. M. (2003). Oxygen sensitivity of NMDA receptors: Relationship to NR2 subunit composition and hypoxia-tolerance of neonatal neurons. *Neuroscience* **118**, 25-35.

Bickler, P. E. and Hansen, B. M. (1998). Hypoxia-tolerant neonatal CA1 neurons: Relationship of survival to evoked glutamate release and glutamate receptor-mediated calcium changes in hippocampal slices. *Dev. Brain Res.* **106**, 57-69.

Boyle, R. (1725). *Philosophical Works*. London: Innys.

Burgard, E. C. and Hablitz, J. C. (1993). Developmental changes in NMDA and non-NMDA receptor-mediated synaptic potentials in rat neocortex. *J. Neurophysiol.* **69**, 230-240.

Cheng, A., Wang, S., Yang, D., Xiao, R. and Mattson, M. P. (2003). Calmodulin mediates brain-derived neurotrophic factor or cell survival signaling upstream of Akt kinase in embryonic neocortical neurons. *J. Biol. Chem.* **278**, 7591-7599.

Cherici, G., Alesiani, M., Pellegrini-Giampietro, D. and Moroni, M. (1991). Ischemia does not induce the release of excitotoxic amino acids from the hippocampus of newborn rats. *Dev. Brain Res.* **60**, 235-240.

Duffy, T. E., Kohle, S. J. and Vannucci, R. C. (1975). Carbohydrate and energy metabolism in perinatal rat brain: relation to survival in anoxia. *J. Neurochem.* **24**, 271-276.

Dunah, A. W., Yasuda, R. P., Wang, Y. H., Luo, J., Davila-Garcia, M., Gbadegesin, M., Vicini, S. and Wolfe, B. B. (1996). Regional and ontogenetic expression of the NMDA receptor NR2D protein in rat brain using a subunit-specific antibody. *J. Neurochem.* **67**, 2335-2345.

Durand, G. M., Kovalchuk, Y. and Konnerth, A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* **381**, 71-75.

Fahlman, C. S., Bickler, P. E., Sullivan, B. and Gregory, G. A. (2002). Activation of the neuroprotective ERK signaling pathway by fructose-1,6-bisphosphate during hypoxia involves intracellular Ca^{2+} and phospholipase C. *Brain Res.* **958**, 43-51.

Franklin, J. L. and Johnson, E. M. J. (1994). Block of neuronal apoptosis by a sustained increase of steady-state free Ca^{2+} concentration. *Phil. Trans. R. Soc. Lond. B* **345**, 251-256.

Friedman, J. and Haddad, G. (1993). Major differences in Ca^{2+}_i responses to anoxia between neonatal and adult CA1 neurons: Role of Ca^{2+}_o and Na^{+}_o. *J Neurosci.* **13**, 63-72.

Gozal, E., Gozal, D., Pierce, W. M., Thongboonkerd, V., Scherzer, J. A., Sachleben, L. R. J., Brittian, K. R., Guo, S. Z., Cai, J. and Klein, J. B. (2002). Proteomic analysis of CA1 and CA3 regions of rat hippocampus and differential susceptibility to intermittent hypoxia. *J. Neurochem.* **83**, 331-345.

Haddad, J. J. and Land, S. C. (2000). O_2-evoked regulation of HIF-1α and NF-κB in perinatal lung epithelium requires glutathione biosynthesis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **278**, L492-L503.

Hestrin, S. (1992). Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. *Nature* **357**, 686-689.

Hochachka, P. (1986). Defense strategies against hypoxia and hypothermia. *Science* **231**, 234-241.

Hochachka, P., Buck, L., Doll, C. and Land, S. (1996). Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc. Natl. Acad. Sci. USA* **93**, 9493-9498.

Hochachka, P. W. and Guppy, M. (1987). *Metabolic Arrest and the Control of Biological Time*. Cambridge, MA: Harvard University Press.

Ikonomidou, C., Mosinger, J. L., Salles, K. S., Labruyere, J. and Olney, J. W. (1989). Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to *N*-methyl-D-aspartate neurotoxicity. *J. Neurosci.* **9**, 2809-2819.

Kirsen, E. D., Schirra, C., Konnerth, A. and Yaari, Y. (1999). Early postnatal switch in magnesium sensitivity of NMDA receptors in rat CA1 pyramidal cells. *J. Physiol.* **521**, 99-111.

Lampe, P. A., Cornbrooks, E. B., Juhasz, A., Johnson, E. M., Jr and Franklin, J. L. (1995). Suppression of programmed neuronal death by a thapsigargin-induced Ca^{2+} influx. *J. Neurobiol.* **26**, 205-212.

Lee, J.-M., Zipfel, G. J. and Choi, D. W. (1999). The changing landscape of ischaemic brain injury mechanisms. *Nature* **399**, A7-A14.

Lutz, P. L. and Milton, S. L. (2004). Negotiating brain anoxic survival in the turtle. *J. Exp. Biol.* **207**, 3141-3147.

Lutz, P. L., Nilsson, G. E. and Prentice, H. M. (2003). *The Brain Without Oxygen: Causes of Failure-Physiological and Molecular Mechanisms for Survival*. Dordrecht, Boston, London: Kluwer Academic Publishers.

Marks, J. D., Friedman, J. E. and Haddad, G. G. (1996). Vulnerability of CA1 neurons to glutamate is developmentally regulated. *Dev. Brain Res.* **97**, 194-206.

Mattson, M. P. (1997). Neuroprotective signal transduciton: Relevance to stroke. *Neurosci. Biobehav. Rev.* **21**, 193-206.

Millhorn, D., Beitner-Johnson, D., Conforti, L., Conrad, P., Kobayashi, S., Yuan, Y. and Rust, R. (2000). Gene regulation during hypoxia in excitable oxygen-sensing cells: depolarization-transcription coupling. *Adv. Exp. Med. Biol.* **475**, 131-142.

Milton, S. L., Thompson, J. W. and Lutz, P. L. (2002). Mechanisms for

maintaining extracellular glutamate levels in the anoxic turtle striatum. *Am. J. Physiol. Regul. Int. Comp. Physiol.* **282**, R1317-1323.

Minet, E., Arnould, T., Michel, G., Roland, I., Mottet, D., Raes, M., Remacle, J. and Michiels, C. (2000). ERK activation upon hypoxia: involvement in HIF-1 activation. *FEBS Lett.* **468**, 53-58.

Mottet, D., Michel, G., Renard, P., Ninane, N., Raes, M. and Michiels, C. (2003). Role of ERK and calcium in the hypoxia-induced activation of HIF-1. *J. Cell Physiol.* **194**, 30-44.

Nicole, O., Ali, C., Docagne, F., Plawinski, L., MacKenzie, E. T., Vivien, D. and Buisson, A. (2001). Neuroprotection mediated by glial cell line-derived neurotrophic factor: involvement of a reduction of NMDA-induced calcium influx by the mitogen-activated protein kinase pathway. *J. Neurosci.* **21**, 3024-3033.

Pei, L., Wiser, O., Slavin, A., Mu, D., Powers, S., Jan, L. Y. and Hoey, T. (2003). Oncogenic potential of TASK3 (Kcnk9) depends on K^+ channel function. *Proc. Natl. Acad. Sci. USA* **100**, 7803-7807.

Prabhakar, N. R. and Overholt, J. L. (2000). Cellular mechanisms of oxygen sensing at the carotid body: heme proteins and ion channels. *Respir. Physiol.* **122**, 209-221.

Puka-Sundvall, M., Sandberg, M. and Hagberg, H. (1997). Brain injury after hypoxia-ischemia in newborn rats: relationship to extracellular levels of excitatory amino acids and cysteine. *Brain Res.* **750**, 325-328.

Rice, M. E., Lee, E. J. and Choy, Y. (1995). High levels of ascorbic acid, not glutathione, in the CNS of anoxia-tolerant reptiles contrasted with levels in anoxia-intolerant species. *J. Neurochem.* **64**, 1790-1799.

Semenza, G. L. (1999). Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. *Annu. Rev. Cell Dev. Biol.* **15**, 551-578.

Soppini, L., Buchs, P. A. and Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *J. Neurosci. Methods* **37**, 173-182.

Tovar, K. R. and Westbrook, G. L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* **19**, 4180-4188.

Wang, Y. H., Bosy, T. Z., Yasuda, R. P., Grayson, D. R., Vicini, S., Pizzorusso, T. and Wolfe, B. B. (1995). Characterization of NMDA receptor-specific antibodies: distribution of NR2A and NR2B receptor subunits in rat brain and ontogenetic profile in the cerebellum. *J. Neurochem.* **65**, 176-183.

Wenzel, A., Villa, M., Mohler, H. and Benke, D. (1996). Developmental and regional expression of NMDA receptor subtypes containing the NR2D subunit in rat brain. *J. Neurochem.* **66**, 1240-1248.

Zhong, J., Carrozza, D. P., Williams, K., Pritchett, D. B. and Molinoff, P. B. (1995). Expression of mRNAs encoding subunits of the NMDA receptor in developing rat brain. *J. Neurochem.* **64**, 531-539.