The Journal of Experimental Biology 205, 3579–3586 (2002)
Printed in Great Britain © The Company of Biologists Limited
JEB4237

Commentary

Adaptive responses of vertebrate neurons to hypoxia

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Accepted 23 August 2002

Summary

The damage caused to mammalian neurons during ischaemic events in the brain (e.g. following a stroke), is an area of major interest to neuroscientists. The neurons of hypoxia-tolerant vertebrates offer unique models for identifying new strategies to enhance the survival of hypoxia-vulnerable neurons. In this review, we describe recent advances in our understanding of how hypoxia-tolerant neurons detect decreases in oxygen and create signals that have immediate and long-term effects on cell function and survival. Sensing and adapting to low oxygen tension involves numerous modalities with different times of activation and effect. Sensors include membrane proteins such as ionotropic ion channels, membrane or cytosolic heme proteins, mitochondrial proteins and/or oxygen sensitive transcription factors such as HIF-1α and NFκB. Signaling molecules involved in O_2 sensing include mitogen-activated protein kinases, ions such as Ca^{2+} and metabolites such as adenosine. These signals act rapidly to reduce the conductance of ion channels (ion flux arrest) and production of energy (metabolic arrest), and slowly to activate specific genes. The ability to construct an energy budget, illustrating which physiological processes are depressed during both long-term and acute metabolic suppression in hypoxia-tolerant neurons, would be of significant value in devising new strategies for neuroprotection. Additionally it is not known how metabolism is regulated at 'pilot-light' levels at which energy-producing and energy-consuming processes are balanced. The regulation of organelle and cell fate during long-term hypoxia is almost completely unexplored, and whether programmed cell death and regeneration of lost neurons occur following protracted dormancy is also of considerable interest.

Key words: neuron, hypoxia, oxygen sensor, intracellular calcium, vertebrate.

Introduction

Neurons are generally viewed as among the most anoxia-sensitive of all cells. However, recent studies have clearly shown that even the most vulnerable neurons are not defenseless, and the most tolerant show a spectacular ability to live without oxygen for prolonged periods (Hochachka and Lutz, 2001; Nilsson, 2001).

Neurons vary widely in their capacity to adapt to a limited oxygen supply to the brain, reflecting the diversity of neuronal functions and the degree of hypoxia normally experienced. This review will define some of the major gaps in our understanding of this adaptive ability in vertebrate neurons. We will specifically review evidence for cellular oxygen sensors, ion channel arrest and metabolic suppression, and oxygen-sensitive signal transduction processes that modulate survival. As will be apparent, huge gaps remain in our knowledge of these adaptations and abundant opportunities exist to ameliorate this knowledge deficit.

Examining the responses of hypoxia-tolerant neurons to decreased oxygen availability is of interest for several reasons. First, oxygen signaling is well developed in hypoxia-tolerant neurons, making them ideal models for studying signal transduction processes during adaptations to hypoxia. Second, hypoxia-tolerant neurons are useful models for distinguishing between injury and adaptation induced by hypoxia. This is of obvious interest in determining the relevance of proposed therapeutic interventions for patients with hypoxic or ischemic diseases. Third, these neurons may help in the identification of entirely new targets for treating diseases that involve hypoxia.

Where are hypoxia-tolerant neurons found?

Hypoxia-tolerant neurons are found in every Order of vertebrates, but most of the experimental work has involved just a few species. Probably the best characterized hypoxia-tolerant neurons are those from the cerebrocortex of the freshwater turtle genus *Chrysemys* (painted turtles). The western painted turtle *Chrysemys picta* survives 5 months of anoxia at 1–3°C during winter dormancy. At warmer

temperature this species tolerates anoxia 100–1000 times longer than mammals (Lutz, 1992; Lutz and Nilsson, 1997a; Ultsch and Jackson, 1982). Interestingly, many turtle species are probably not particularly tolerant of anoxia (Crocker et al., 1999), suggesting that adaptation for prolonged lack of oxygen does not automatically come with having a shell! Neurons from turtles represent an extreme; these cells survive anoxia by profoundly reducing all aspects of their activity, and under these conditions the majority of neurons must be functionally 'switched off' for long periods of time.

Hypoxia-tolerant fishes such as carp contrast with turtles in that their neurons apparently maintain a higher state of vigilance during anoxia, i.e. the brain remains active (Nilsson, 2001). Other fishes, such as estivating African lungfish, enter dormant, suspended animation states during dry periods, which are possibly associated with at least some degree of hypoxia. Numerous fish species reside in the oxygen-depleted waters of the Amazon basin and these species offer exciting opportunities for exploration…

Adult amphibians may experience low ambient oxygen during retreats to burrows, cocoons or other refugia during dry seasons (e.g. the Australian frog *Cyclorana platycephala* or the American spadefoot toad *Scaphiopus couchii*), although the degree of hypoxia they encounter is probably not extreme. Spadefoot toads decrease metabolism substantially during dormancy and some information is available on the regulation of their hypometabolic condition (Storey, 2002). Species that spend the winter dormant in hypoxic water have also been of interest. The protective effect of metabolic rate depression during cold hypoxic submergence has been demonstrated in adult hibernating *Rana temporaria* (Donohoe et al., 2000). *Rana* brain maintains ATP levels for 2 days at 3°C in anoxia before declining (P. H. Donohoe and R. G. Boutilier, unpublished data). Throughout all stages of prolonged hypoxia (water P_{O_2} 30–60 mmHg), for up to 16 weeks at 3°C, frog brain ATP levels were maintained, although briefer anoxia at 25°C is associated with ATP loss (Knickerbocker and Lutz, 2001). The ability to depress metabolic rate such that ATP demands can be met by oxidative phosphorylation in an oxygen-limited environment is probably the key to the frogs' overwintering survival. Tadpoles may be good models for studies on the effects of hypoxia on the brain because some species are hypoxia tolerant (West and Burggren, 1982) and the cells of the central nervous system are relatively accessible for study.

Numerous mammals are hypoxia tolerant. The naked Kenyan mole rat *Hetercephalus glaber*, which lives in a burrow environment of approx. 8% oxygen, is a noteworthy example. Synaptic transmission in the mole rat hippocampus is very tolerant of low oxygen and recovers quickly from anoxia (J. Larsen and T. Park, unpublished data). Cortical and hippocampal neurons from oxygen-sensitive species such as *Rattus norvegicus* are hypoxia-tolerant during the embryonic and neonatal periods (Adolph, 1948; Bickler and Hansen, 1998; Haddad and Donnelley, 1990; Haddad and Jiang, 1993). This is not surprising because oxygen tension in fetal brain is less than half the normal value for adults (Parer, 1993). In some species the birth process or the nesting environment may be associated with hypoxia. Mammalian hibernation is associated with tolerance of hypoxia or ischemia, although hibernation is neither ischemic or hypoxic, since blood flow and metabolism are decreased in parallel and lactic acid does not accumulate (Hochachka and Guppy, 1987). Neurons from hibernating ground squirrels tolerate hypoxia-glucose deprivation even when studied at 37°C, suggesting that hypothermia is but one factor in the tolerance of this tissue (Frerichs, 1999).

Little is known mechanistically concerning the adaptation of marine mammals to the brain hypoxia that accompanies deeper, long-duration dives, even though their tolerance is well defined (Elsner et al., 1972; Lutz and Nilsson, 1997b). Hypometabolism occurs during diving in seals (Hurley and Costa, 2001) and it would be fascinating to determine if portions of the brain participate in energy savings by undergoing a reduction in activity during long dives.

Why does the lack of O_2 kill typical neurons rapidly?

Hypoxia kills neurons because anaerobic energy production fails to keep pace with demands. The 'critical oxygen tension', the P_{O_2} at which brain ATP production in adult mammals begins to decline, is between 25 and 40 mmHg (3.29–5.33 kPa) (Erecinska and Silver, 2001). Many types of hypoxia-tolerant neurons solve this risk of energy failure not by increasing anaerobic metabolism, but by decreasing the demand for energy sufficiently to prevent the loss of high-energy metabolic intermediates such as ATP and phosphocreatine (Hochachka, 1986). Understanding this response offers great promise in the hunt to identify mechanisms that control and coordinate a hypometabolic strategy of anoxic survival.

When oxygen or blood flow to the mammalian brain decreases to critical levels, energy failure occurs, with a decline in ATP by as much as 90% in as little as 5 min (Erecinska and Silver, 2001). When 50–65% of the ATP is lost, depolarization of the membrane and subsequent uptake of sodium and water occurs (Hansen, 1985; Knickerbocker and Lutz, 2001). Depolarization causes Ca^{2+} influx through voltage-gated Ca^{2+} channels. The Na^+ gradient collapse causes the sodium-glutamate cotransporters to eject glutamate into the extracellular space (Rossi et al., 2000). Glutamate triggers vigorous activation of glutamate receptors, initiating a process of calcium influx and excitatory injury called the glutamate cascade. *N*-methyl-D-aspartate glutamate receptors are responsible for a significant part of the Ca^{2+} influx and the Ca^{2+}-dependent cell injury that ensue (Lee et al., 1999). Glutamate receptors have been primary targets for experimental treatment of ischemic brain injury (Choi, 1995; Lee et al., 1999). The death of neurons from these insults can follow quickly from swelling and lysis (necrosis) or evolve over many days. This slower cell death is complex and in some respects resembles programmed cell death or apoptosis. An excellent review of the role of programmed cell death in brain ischemia was recently published (Lipton, 1999).

A most fundamental adaptive response of hypoxia-tolerant

cells to oxygen lack is the capacity to avoid a drastic decline in ATP levels at a time of absent aerobic ATP production. It is likely that similar responses to energetic stress are found in cells during hibernation and estivation, although hypoxia does not characterize these states. A drastic, balanced, suppression of ATP demand and supply pathways must occur in all these conditions; this regulation allows ATP levels to remain relatively constant, even while ATP turnover rates greatly decline. In neurons, the ATP requirements of ion pumping (mainly Na^+; Rolfe and Brown, 1997) are downregulated by 'channel' arrest (Bickler et al., 2002). The ATP demands of protein synthesis also must be downregulated, and although rapid and global suppression of protein synthesis occurs in anoxia-adapted hepatocytes (Hochachka et al., 1996), we think it likely that selective rather than global suppression of gene expression and protein synthesis occurs in neurons. This necessitates that a significant percentage of the neurons in the brain are functionally inactivated during anoxia, therefore cannot participate in vigilance or regulation activities. Accordingly, 'metabolic arrest' cannot be the only cellular response for vertebrate neurons – some neuron groups must remain 'vigilant' and participate in regulation and eventual arousal from dormancy/inactivity. Indeed, a report by Fernandes et al. (1997) shows that electroencephalograph activity in anoxic turtles waxes and wanes over periods of many hours.

Sensing the lack of oxygen

Multiple O_2 sensors are important in adapting neurons to hypoxia. Some of these modalities are illustrated in Fig. 1. In some cases O_2 may interact directly with a target molecule that influences neuronal function. O_2-sensitive sodium channels and potassium channels are examples that produce rapid adjustments in neuronal excitability. Usually the actions of hypoxia on ion channels appear to be adaptive, in that the effect is suppression of excitatory channels and augmentation of inhibitory ones. However, at least one type of sodium channel is activated by hypoxia (Table 1); it is possible that this effect is responsible for initiating other types of protective responses such as augmentation of behavioral or physiological compensatory mechanisms to lack of O_2.

Some of the effects of O_2 on ion channels may be mediated by membrane proteins containing heme groups or redox-sensitive sites such as NADPH oxidase (Prabhakar and Overholt, 2000), where the redox state of the oxidase controls ion flux or other effects (Fig. 1).

Perhaps the best-described O_2-sensing pathway is the

Table 1. *Effects of hypoxia on neuronal ion channels*

Channel type	Effect of hypoxia	Mechanism of effect	References
Voltage-gated channels			
Potassium			
TASK1[1]	Inhibition	Direct effect on channel protein (?)	Lewis et al., 2001
K_{Ca}	Activation	↑ $[Ca^{2+}]_i$ activates channel	Porter et al., 2001
Kv[2] (7 subtypes)	Inhibition	Direct effect on channel proteins?	Patel and Honore, 2001
Sodium			
Voltage-dependent	Potentiation of current	Redox modulation by membrane associated protein (stimulation)	Hammarstrom and Gage, 2000; Perez-Pinzon et al., 1992
Non-inactivating	Activation or inhibition	Removal of channels from membrane	
Calcium			
T-type	Inhibition	Redox site on associated sensor?	Fearon et al., 2000
L-type	Inhibition	Redox site on associated sensor?	Fearon et al., 1999
Ligand-gated channels			
NMDA-type glutamate receptors	Inhibition	Removal from membrane Dephosphorylation Ca^{2+}-dependent inactivation H^+/Mg^{2+} inhibition adenosine	Bickler et al., 2000; Buck and Bickler, 1998
Conductance channels			
Whole cell conductance in turtle neurons	Inhibition	Depends on ↑ $[Ca^{2+}]_i$	Ghai and Buck, 1999

Removal of sodium channels from the membrane, modulation of NMDA receptors, and closure of conductance channels were demonstrated in hypoxia-tolerant neurons.

The other effects listed may occur in both hypoxia-sensitive and hypoxia-tolerant cells.

[1]TASK, tandem pore, acid-sensitive potassium channel.

[2]Kv, single pore, voltage-gated potassium channel.

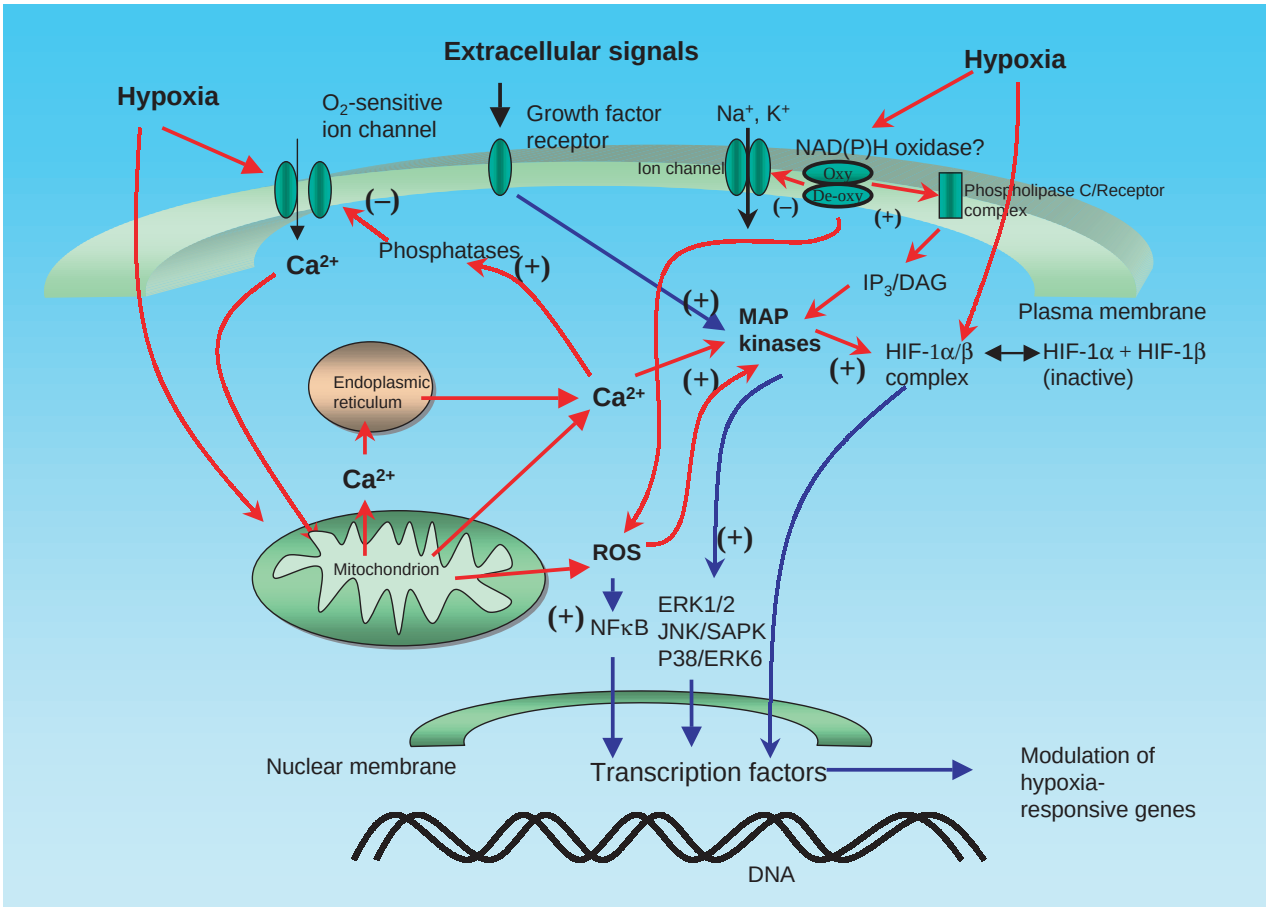

Fig. 1. Oxygen signaling in neurons. Rapid responses to hypoxia are shown in red and more slowly developing responses are shown in blue. (+) indicates a potentiating effect on the target, (–) an inhibitory one. Oxygen interacts with a variety of target molecules, both at the cell surface, e.g. ion channels (see Table 1), NADPH oxidase (Prabhakar and Overholt, 2000), cytosol, e.g. HIF and related proteins (Semenza, 1999), and organelles such as mitochondria. Decreases in oxygen tension have direct effects on some ion channels (e.g. potassium channels, see references in Table 1) and on molecules associated with transcription factors such as HIF-1α. Hypoxia has indirect effects mediated by changes in the bioenergetic state of mitochondria *via* intermediate signaling modalities such as Ca^{2+} (Berridge et al., 2000; Bickler et al., 2000) and reactive oxygen species (ROS) (Haddad and Land, 2000). Growth factors (Nicole et al., 2001), cytokines and inorganic ions (Millhorn et al., 2000) may also modulate neuronal responses to hypoxia. Many of these signals converge on MAP kinase cassettes including the ERK, JNK and p38 pathways (Mattson, 1997; Minet et al., 2000; Semenza, 1999).

oxygen-sensitive transcription factor HIF, which is regulated by a series of at least two oxygen-sensitive hydroxylases (Lando et al., 2002). HIF has been reviewed recently (Semenza, 1999) and will not be extensively discussed here.

A less studied hypoxia-modulated transcription factor is NFkB, which may be activated both by extracellular signals such as cytokines and intracellular signals such as reactive oxygen (ROS) (Haddad and Land, 2000). Oxygen sensors associated with heme proteins such as cytochrome *c* in the mitochondria may respond to hypoxia by altering the rate of release of reactive oxygen species that activate transcription factors (Bunn and Poyton, 1996; Vanden Hoek et al., 1998).

A convergent pathway for the regulation of multiple modalities involved in O_2 sensing is the mitogen-activated protein kinase system (MAPK), which is composed of different cassettes terminating in a variety of transcription factors. Interestingly, the MAPK ERK1/2 modulates HIF-1a by posphorylation control (Minet et al., 2000). Extracellular growth factor signals strongly influence the activity of HIF, probably through the MAPK system (Semenza, 1999).

Recent evidence suggests that membrane-bound phospholipase C may play a role in transducing the signal of hypoxia into alterations in the phosphorylation state of MAP kinase. In our laboratory, we found that phosphorylation of the MAPK kinase p42/42 (associated with neuroprotective gene expression induced by agents such as growth factors) occurs during hypoxia and that it depends on phospholipase C and intracellular calcium (Donohoe et al., 2001).

In summary, oxygen sensing is achieved by a variety of molecules whose effects are complexly intertwined. Evidence exists for rapid and longer-term effects of such signaling, as described below.

Putting the signal for lack of oxygen to work

In hypoxia-tolerant neurons a decline in O_2 availability leads to a series of adaptations, including: (1) a reduction in ion movements across the neurolemma; (2) a balanced reduction in energy production and energy consumption; and (almost certainly) (3) specific gene expression. Below we discuss how each of these is achieved, dividing the responses into immediate and long-term.

Acute responses to hypoxia

Direct and indirect modulation of ion channel activity

The following mechanisms, documented in Table 1, are known to regulate ion channels in hypoxic neurons (Bickler et al., 2000; Prabhakar and Overholt, 2000). (1) Modulation of the channel by redox sites on the channel protein or direct oxygen interaction with the channel protein. (2) Phosphorylation/dephosphorylation of channel proteins by kinases and phosphatases. (3) Ca^{2+}-dependent regulation *via* the cytosleleton. (4) Modulation by extracellular ions such as H^+ or Mg^{2+}. (5) Regulation by neuromodulators, such as adenosine, which act through G-proteins. A slower mechanism involves removal and re-insertion of ion channel molecules from the cell membrane (Bickler et al., 2000).

An important feature of these controls is that they operate over a wide range of time scales and are thus suited to variations in the duration of hypoxia.

Some oxygen-sensitive ion channels may have oxygen sensors directly associated with the channel. In the rat kVβ2 channel the β subunit is an oxidoreductase that includes a nicotinamide cofactor (Bahring et al., 2001). The oxidoreductase apparently s directly with the voltage sensing portion of the channel, influencing its activity depending on oxygen tension. Table 1 lists some known effects of oxygen on ion channel regulation.

Acute responses: Interaction of bioenergetics and $[Ca^{2+}]_i$

Intracellular calcium plays a major role in controlling many cellular processes (e.g. synaptic plasticity, neurotransmitter release, cell proliferation and cell survival; for review, see Berridge et al., 2000). At the subcellular level, calcium acts as a second messenger, which modulates processes such as the release of intracellular calcium stores, gene transcription and function of protein kinases and phosphatases. Transient increases in $[Ca^{2+}]_i$ are required for normal cell functioning, but sustained increases in $[Ca^{2+}]_i$ above 200 nmol l^{-1} are usually considered pathological in neurons (Lee et al., 1999).

Mitochondria contribute to $[Ca^{2+}]_i$ homeostasis by acting as a calcium source or ‘sink’ when cytosolic calcium reaches inadequate or excessive levels. Mitochondrial Ca^{2+} homeostasis is tightly coupled to ATP production during hypoxia because Ca^{2+} accumulates in the matrix when electron transport is uncoupled from oxidative phosphorylation. Calcium accumulation contributes to the depolarization of the mitochondrial membrane potential.

Depolarisation-transcriptional coupling

Ca^{2+} is also an important regulator of gene expression. Global elevations of $[Ca^{2+}]_i$, acting *via* calmodulin, control the rapid gene expression triggered by neuronal depolarisation (Millhorn et al., 2000). Hardingham et al. (2001) suggest that a second, rapidly responding, excitation–transcription transduction pathway exists in neurons, whereby $[Ca^{2+}]$ microdomains, located proximal to NMDA receptors, remotely affect nuclear $[Ca^{2+}]$, which is the central regulator of transcription. They demonstrate that the discrete elevation in $[Ca^{2+}]$, restricted to the site of entry in the synapse, is propagated independently of a global elevation in $[Ca^{2+}]$ to the nucleus by an extracellular signal-regulated kinase (ERK1/2).

Acute suppression of metabolism

Survival of long-term anoxia requires that neurons decrease their substrate utilization so as to avoid energy depletion. In neurons, little is known about how this is done. Much more is known about regulation of substrate utilization in dormant, aerobic states such as estivation in land snails or in hibernators. Studies in these non-hypoxic but metabolically quiescent animals have clearly shown that hypometabolism is associated with phosphorylation control of glycolytic enzymes (Storey, 2002). Whether this principle applies during hypoxic dormancy has not been determined, but it seems reasonable that it does. The signals that initiate the activation of protein kinases or phosphatases have not been identified. Phosphorylation controls are probably but one aspect of a hypometabolic state in dormant animals.

Bishop et al. (2001) have examined how dormancy influences mitochondrial function in land snail hepatopancreas cells. They observed that 75% of the decline in oxidative respiration observed during aestivation is due to a drop in oxidative respiratory enzyme kinetics, i.e. a fall in activity of the proteins that produce the mitochondrial membrane potential ($\Delta\Psi_m$). The lowered $\Delta\Psi_m$ results in a subordinate decline in the oxidative respiration driving ATP production.

Long-term responses: transcriptional regulation

Hypoxia induced gene expression

A large literature exists on the role of the HIF-1α transcription factor in the expression of genes such as erythropoetin, which enhance oxygen delivery to tissues (Semenza, 1999). Hypoxia also increases the expression of genes whose products facilitate rearrangements of metabolism for optimal function during hypoxia. For example, hypoxic preconditioning (8% O_2 for 3 h), a treatment known to protect the newborn rat brain against hypoxic–ischemic injury, markedly increases HIF-1α and HIF-1β expression. Preconditioning with hypoxia 24 h before a hypoxia–ischemia insult affords very substantial reduction of brain infarction in rodents (Bergeron et al., 1999). Coincidentally, hypoxic preconditioning increased the expression of two glucose transporters (GLUT-1, GLUT-3) and 11 glycolytic enzymes. This suggests that the modulation of glucose transport and

glycolysis by hypoxia may contribute to the development of hypoxia-induced tolerance. The specific roles of hypoxia-augmented transcription factors such as HIF-1α in hypoxia-tolerant neurons have yet to be explored.

Are responses to hypoxia adaptive or injurious?

One advantage of studying hypoxia-tolerant cells is that they enable the identification of protective responses; in hypoxia-intolerant neurons the effects of oxygen-lack are often a confusing mixture of injury and adaptive responses. For example, turtle cerebrocortical neurons are very tolerant of anoxia, even though their intracellular calcium $[Ca^{2+}]_i$ doubles within several hours. It can be surmised that this increase in $[Ca^{2+}]_i$ serves a protective or signaling role rather than an injurious one. That an increase in $[Ca^{2+}]_i$ might be protective under some circumstances would have been a difficult concept to develop from studying adult mammalian neurons. The lessons learned from similar observations may provide new insights into treating brain injury from anoxia or stroke.

Hypoxia-tolerant neurons have much to teach about the tolerable limits of alterations in energy state and cellular homeostasis simple because deleterious changes develop slowly and are thus easier to observe. For example, ATP loss from anoxic frog brain evolves over hours to days, not minutes, making events associated with it easy to chronicle (Knickerbocker and Lutz, 2001). New insights can also be obtained about events that may be part of an injury process or part of survival. A good example is the complex process of apoptosis. In recent studies in our laboratory, we found that pro-apoptotic proteins such as Bax are expressed in the turtle brain during prolonged, but survivable, hypoxia. This raises the possibility that either proteins such as Bax actually have a protective side to them or that certain neurons are deciduous – they may die during anoxic dormancy and regenerate in the spring with resumption of aerial respiration. Death of cells almost certainly occurs in many tissues of the body because the mortality from months of hypoxia is large – perhaps up to 30% of turtles die each year during dormancy. At the same time, inhibition of apoptosis may be important to other neurons during dormancy, despite conditions that might otherwise be associated with the triggering of cell death processes. The status of regeneration in the turtle brain following prolonged dormancy will be fascinating to study.

The role of the MAPK signaling system in the regulation of apoptosis is also fertile ground for study. MAPKs regulate the balance between cell survival/differentiation and cell death/apoptosis. The p42/p44 MAPKs (involved in the ERK pathway) is activated by processes that promote neuron survival (e.g. by neuroprotective growth factors such as BDNF; Nicole et al., 2001). In contrast, the p38 pathway is associated with neuron death. Notably, the p42/p44 (also termed ERK) pathway is activated by small increases in $[Ca^{2+}]_i$ during survivable degrees of hypoxia (Minet et al., 2000).

The importance of recovering well from lack of oxygen

Reactive oxygen species (ROS) are generated during hypoxia and especially during re-oxygenation. While ROS exert important controls on transcription factors such as NFκB, which might be protective (Fig. 1), they have substantial potential to contribute to cell injury. A major source of ROS is the leakage of electrons from the electron transport chain, and from enzymes including nitric oxide synthase, xanthine oxidase, lipogenase, cyclooxygenase and epoxygenase (reviewed by Erecinska and Silver, 2001). Re-oxygenation is a critical time since production of free radicals increases dramatically. Some information about defense mechanisms for dealing with free radicals by hypoxia-tolerant organisms is available. One might expect robust ROS-defense mechanisms in hypoxia-tolerant neurons and some evidence suggest that this is the case. Turtle brain has very high concentrations of antioxidant compounds such as ascorbate (Rice et al., 1995), and antioxident defences are high in freeze- and anoxia-tolerant garter snakes (Hermes-Lima and Storey, 1996). Similar defenses may exist in diving marine mammals.

Little is known concerning the issue of how cells in a profound state of dormancy can resume normal activities when favorable environmental conditions return. One hypothesis is that some groups of neurons remain in a vigilant state and are capable of arousing others when appropriate. The signaling events which orchestrate these processes are largely unstudied.

Major unanswered questions

Much remains to be learned about how cells sense and adapt to conditions of oxygen scarcity. Research is needed to describe the diversity of oxygen sensors and the mechanisms by which the sensors activate protective events. It is of particular interest to understand how hypoxia is used to signal a decrease in metabolism, and how hypoxia inactivates the ion channels that would otherwise be involved in triggering excitotoxic death during metabolic stasis. The regulation of gene expression during hypoxia is another area that is not well understood, including what genes are expressed during hypoxia and which are inactivated. The control of cell fate, and the regulation of cell suicide genes and the process of apoptosis, will also be a fascinating story to unravel. Major questions remain concerning the vigilance mechanisms used by animals such as turtles as they traverse months of hypothermia and anoxia – the mechanisms that allow them to recover, avoid the flood of free radicals, and replace injured brain cells.

References

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

Bahring, R., Milligan, C., Vardanyan, V., Engeland, B., Young, B., Dannenberg, J., Waldschutz, R., Edwards, J., Wray, D. and Pongs, O. (2001). Coupling of voltage-dependent potassium channel inactivation and

oxidoreductase active site of Kvbeta subunits. *J. Biol. Chem.* **276**, 22923-22929.

Bergeron, M., Yu, A. Y., Solway, K. E., Semenza, G. L. and Sharp, F. R. (1999). Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain. *Eur. J. Neurosci.* **11**, 4159-4170.

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., 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., Donohoe, P. H. and Buck, L. T. (2002). Molecular adaptations for survival during anoxia: lessons from lower vertebrates. *Neurosci.* **8**, 234-242.

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.

Bishop, T., St.-Pierre, J. and Brand, M. (2001). Primary causes of decreased mitochondrial oxygen consumption during metabolic suppression in snail cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R372-R382.

Buck, L. T. and Bickler, P. E. (1998). Adenosine and anoxia reduce *N*-methyl-D-aspartate receptor open probability in turtle cerebrocortex. *J. Exp. Biol.* **210**, 289-297.

Bunn, H. and Poyton, R. (1996). Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* **76**, 839-885.

Choi, D. W. (1995). Calcium: still center stage in hypoxic-ischemic neuronal death. *Trends Neurosci.* **18**, 58-60.

Crocker, C., Ultsch, G. and Jackson, D. C. (1999). The physiology of diving in a north-temperate and three tropical turtle species. *J. Comp. Physiol. B* **169**, 249-255.

Donohoe, P. H., Fahlman, C. S., Bickler, P. E., Vexler, Z. S. and Gregory, G. A. (2001). Neuroprotection and intracellular Ca^{2+} modulation with fructose-1,6-bisphosphate during in vitro hypoxia-ischemia involves phospholipase C-dependent signaling. *Brain Res.* **917**, 158-166.

Donohoe, P. H., West, T. G. and Boutilier, R. G. (2000). Factors affecting membrane permeability and ionic homeostasis in the cold-submerged frog. *J. Exp. Biol.* **203**, 405-414.

Elsner, R., Shurley, J. T., Hammond, D. D. and Brooks, R. E. (1972). Cerebral tolerance to hypoxemia in asphyxiated Weddell seals. *Resp. Physiol.* **9**, 287-297.

Erecinska, M. and Silver, I. A. (2001). Tissue oxygenation and brain sensitivity to hypoxia. *Resp. Physiol.* **128**, 263-276.

Fearon, I. M., Palmer, A. C., Balmforth, A. J., Ball, S. G., Varadi, G. and Peers, C. (1999). Modulation of recombinant human cardiac L-type Ca^{2+} channel alpha1C subunits by redox agents and hypoxia. *J. Physiol.* **514**, 629-637.

Fearon, I. M., Randall, A. D., Perez-Reyes, E. and Peers, C. (2000). Modulation of recombinant T-type Ca^{2+} channels by hypoxia and glutathione. *Pflugers Arch - Eur. J. Physiol.* **441**, 181-188.

Fernandes, J. A., Lutz, P. L., Tannenbaum, A., Todorov, A. T., Liebovitch, L. and Vertes, R. (1997). Electroencephalogram activity in the anoxic turtle brain. *Am. J. Physiol.* **273**, R911-R919.

Frerichs, K. U. (1999). Neuroprotective strategies in nature – novel clues for the treatment of stroke and trauma. *Acta Neurochirugica Suppl.* **73**, 57-61.

Ghai, H. S. and Buck, L. T. (1999). Acute reduction in whole cell conductance in anoxic turtle brain. *Am. J. Physiol.* **277**, R887-R893.

Haddad, G. and Donnelley, D. (1990). O_2 deprivation induces a major depolarization in brain stem neurons in adult but not in the neonatal rat. *J. Physiol.* **429**, 411-428.

Haddad, G. and Jiang, C. (1993). O_2 deprivation in the central nervous system: On mechanisms of neuronal response, sensitivity, and injury. *Progr. Neurobiol.* **40**, 277-318.

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.

Hammarstrom, A. K. M. and Gage, P. W. (2000). An oxygen-sensing sodium channel in rat hippocampal neurons. *Biophys. J.* **78**, 88A.

Hansen, A. (1985). Extracellular potassium concentration in juvenile and adult brain cortex during anoxia. *Acta Physiol. Scand.* **99**, 412-428.

Hardingham, G. E., Arnold, F. J. L. and Bading, H. (2001). A calcium microdomain near NMDA receptors: on switch for ERK-dependent synapse-to-nucleus communication. *Nature Neurosci.* **4**, 565-566.

Hermes-Lima, M. and Storey, K. B. (1996). Antioxidant defences in the tolerance of freezing and anoxia by garter snakes. *Am. J. Physiol.* **265**, R646-R652.

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. 227pp.

Hochachka, P. W. and Lutz, P. (2001). Mechanism, origin, and evolution of anoxia tolerance in animals. *Comp. Biochem. Physiol. B* **130**, 435-459.

Hurley, J. and Costa, D. (2001). Standard metabolic rate at the surface and during trained submersions in adult California sea lions (*Zalophus californianus*). *J. Exp. Biol.* **204**, 3273-3281.

Knickerbocker, D. L. and Lutz, P. L. (2001). Slow ATP loss and the defense of ion homeostasis in the anoxic frog brain. *J. Exp. Biol.* **204**, 3547-3551.

Lando, D., Peet, D., Whelan, D., Gorman, J. and Whitelaw, M. (2002). Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. *Science* **295**, 858-861.

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

Lewis, A., Hartness, M. E., Chapman, C. G., Fearon, I. M., Meadows, H. J., Peers, C. and Kemp, P. J. (2001). Recombinant hTASK1 is an O_2-sensitive K^+ channel. *Biochem. Biophys. Res. Comm.* **285**, 1290-1294.

Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiol. Rev.* **79**, 1431-1567.

Lutz, P. (1992). Mechanisms for anoxic survival in the vertebrate brain. *Annu. Rev. Physiol* **54**, 601-618.

Lutz, P. and Nilsson, G. (1997a). Contrasting strategies for anoxic brain survival–glycolysis up or down. *J. Exp. Biol.* **200**, 411-419.

Lutz, P. L. and Nilsson, G. E. (1997b). *The Brain Without Oxygen: Causes of Failure and Mechanisms for Survival*. Austin: R. G. Landis. 227pp.

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.

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.

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.

Nilsson, G. E. (2001). Surviving anoxia with the brain turned on. *News Physiol. Sci.* **16**, 217-221.

Parer, J. T. (1993). Fetal uteroplacental circulation and respiratory gas exchange. In *Anesthesia for Obstetrics* (ed. S. M. Shnider and G. Levinson), pp. 19-28. New York: Williams and Wilkins.

Patel, A. J. and Honore, E. (2001). Molecular physiology of oxygen-sensotive potassium channels. *Eur. Resp. J.* **18**, 221-227.

Perez-Pinzon, M., Rosenthal, M., Sick, T., Lutz, P., Pablo, J. and Mash, D. (1992). Downregulation of sodium channels during anoxia: a putative survival strategy of turtle brain. *Am. J. Physiol.* **262**, R712-R715.

Porter, V. A., Rhodes, M. T., Reeve, H. L. and Cornfield, D. N. (2001). Oxygen-induced fetal pulmonary vasodilation is mediated by intracellular calcium activation of K_{Ca} channels. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **281**, L1379-L1385.

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

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.

Rolfe, D. F. S. and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731-758.

Rossi, D. J., Oshima, T. and Attwell, D. (2000). Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* **403**, 316-321.

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

Storey, K. B. (2002). Life in the slow lane: molecular mechanisms of estivation. *Comp. Biochem. Physiol.* (in press).

Ultsch, G. and Jackson, D. (1982). Long-term submergence at 3°C of the turtle *Chrysemys picta belli*, in normoxic and severely hypoxic water. I. Survival, gas exchange and acid–base status. *J. Exp. Biol.* **96**, 11-28.

Vanden Hoek, T., Becker, L., Shao, Z., Li, C. and Schumacker, P. (1998). Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J. Biol. Chem.* **273**, 18092-18098.

West, N. and Burggren, W. (1982). Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole. *Resp. Physiol.* **47**, 165-176.