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Review

Negotiating brain anoxia survival in the turtle

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Summary

The turtle brain's extraordinary ability to tolerate anoxia is based on constitutive and expressed factors. Constitutive factors that predispose for anoxia tolerance include enhanced levels of glycogen stores, increased densities of protective receptors, elevated antioxidant capacities and elevated heat shock protein. However, to survive an anoxic insult, three distinct phases must be negotiated successfully. (1) A coordinated downregulation of ATP demand processes to basal levels. This phase, which takes 1–2 h, includes a reduction in voltage-gated K^+ (Kv) channel transcription and a substantial increase in Hsp72 and Hsc73 levels. During this period, adenosine and K_{ATP} channels mediate several key events including channel arrest initiation and a reduction in the release of excitatory amino acids (EAAs). (2) Long-term survival (days) at basal levels of ATP expenditure. Neuronal network integrity is preserved through the continued operation of core activities. These include periodic electrical activity, an increased release of GABA and a continued release of glutamate and dopamine. Adenosine and GABA modulate the glutamate release. There is a further increase in Hsc73, indicating a 'housekeeping' role for this protein during this period. (3) A rapid upregulation of neuronal processes when oxygen becomes available to restore full function, together with the activation of protection mechanisms against reperfusion-generated reactive oxygen species.

Key words: anoxia tolerance, ATP expenditure, GABA, heat shock protein, turtle, *Trachemys scripta.*

Introduction

There is a vast literature on the effects of anoxia/ischemia on the mammalian brain. In essence, unable to compromise on its intense energy consumption, the mammalian brain starts to lose ATP within minutes of being deprived of oxygen. After a few minutes, when about 65% of the ATP is lost, there is a failure in ATP-dependent ion exchangers (Knickerbocker and Lutz, 2001), resulting in the breakdown of ionic gradients followed by membrane depolarization. Depolarization allows a cytotoxic increase in intracellular Ca^{2+} concentration, the uncontrolled release of excitatory neurotransmitters, such as glutamate, in neurotoxic amounts and subsequent neuronal death (Lipton, 1999). When temperature differences are taken into account, this scenario is characteristic of the brains of vertebrates ranging from fish to mammals (Lutz et al., 2003a).

In contrast to the high vulnerability of the mammalian brain to hypoxia (as well as most other vertebrates), the brain of the freshwater turtle *Trachemys scripta* is able to withstand anoxia for days at room temperature (Lutz et al., 2003a; Bickler et al., 2002). Recent research shows that this ability is not simply one of passive tolerance but is due to a fascinating, interlocking cluster of adaptations that produce a state of deep hypometabolism, the most effective of all hypoxia defense strategies (Hochachka and Lutz, 2001). The current review discusses some recent advances in our understanding of how the turtle more effectively uses some of the early protective mechanisms activated in the mammal and how the turtle brain defends against the catastrophic events of anoxic failure.

Constitutive factors for anoxia survival

Although the turtle brain initiates an elaborate defense strategy when it encounters anoxic conditions, the normoxic brain also has features that predispose it to tolerate hypoxia (a more likely routine event; Lutz, 1992) and that also prime it to assist in immediate survival in an anoxic crisis. Some of these factors include high-energy stores, receptor and enzyme activities that provide protection, and molecular adaptations.

The turtle brain is remarkable in having glycogen concentrations about fivefold greater than that of the rat or the anoxia-intolerant rainbow trout (Lutz et al., 2003a). Presumably, this provides an immediately accessible store of glucose for anaerobic glycolysis until adequate glucose supplies are liberated (recruited) from the large glycogen stores in the liver.

In general, the turtle has much lower aerobic enzyme activities and ion channel densities than the mammal, at levels

that roughly match the difference in their metabolic intensities (Lutz et al., 2003a). For example, the maximal binding capacity (B_{max}) of the turtle brain adenosine A_1 receptor is only 10–20% that of the rat brain (Lutz and Manuel, 1999), Na^+/K^+-ATPase activities are ~40% (Suarez et al., 1989) and the density of voltage-dependent Na^+ channels is ~30% (Edwards et al., 1989). By contrast, some receptors that have protective roles are much more abundant. The turtle appears to have a comparatively high intrinsic inhibitory potential since its cerebral hemispheres have the same density of inhibitory $GABA_A$ receptors as the rat (Lutz and Kabler, 1995). Moreover, Xia and Haddad (2001) found that the B_{max} for the δ-opioid receptor in the turtle cortex is more than four times that of the rat. As δ-opioid receptors protect neurons against glutamate, they speculate that the turtle brain may be pre-protected from glutamate excitotoxicity (Xia and Haddad, 2001). Indeed, there is evidence that turtle brain is indeed more resistant to glutamate toxicity (Wilson and Kreigstein, 1991). The level of the pituitary adenylate cyclase activating polypeptide, PACAP 38, may be the most striking example of constitutive protection, being 10–100-fold higher in the turtle brain than in rat and human brain (Reglodi et al., 2001). PACAP has a neuroprotective role in ischemia, and there is evidence that the high levels may help protect against anoxia-induced neuronal damage in the turtle retina (Rabl et al., 2002).

Heat shock proteins are an important class of molecular adaptations that protect against physiological stressors. They act as molecular chaperones, protecting against the denaturation of proteins or refolding unfolded proteins, and those associated with the Hsp70 family are particularly important in providing protection from hypoxia-related damage (Snoeckx et al., 2001). One member, Hsc73, is regarded as a cognate or constitutive protein because it is present in non-stressed tissues and is only slightly inducible by stress (Snoeckx et al., 2001). The inducible heat shock protein Hsp72 is hardly seen in unstressed conditions in the mammalian brain (Snoeckx et al., 2001). By contrast, in the turtle brain, both Hsp72 and Hsc73 are found at high levels in normoxia (Prentice et al., in press). Interestingly, the inducement of Hsp72 by a short mild exposure to ischemia/hypoxia is thought to be an important feature of preconditioning, providing temporary protection for subsequent otherwise injurious ischemic/anoxic insults (Marber et al., 1993). In this regard, the high constitutive levels of Hsp72 in normoxic turtle brain indicate that the turtle has an element of preconditioning already expressed.

In the mammal, nuclear factor κB (NF-κB) is an important transcription regulator of many genes that play a role in recovery from acute or chronic trauma (Haddad, 2002). In particular, it is central to the expression of stress-responsive genes in the face of inflammatory and oxidative damage, protects against reactive oxygen species (ROS) damage and has an anti-apoptotic function (Haddad, 2002). The turtle has high normoxic levels of NF-κB (Lutz and Prentice, 2002), which might correspond to a constitutive pro-survival state.

Finally, the freshwater turtle brain may show an enhanced predisposition to fight reoxygenation damage. The turtle brain has greater concentrations of ascorbic acid compared with mammals, levels in cortex being 2–3 times greater than in the mammalian cortex (Rice et al., 1995). The freshwater turtle also has high constitutive activities of catalase, superoxide dismutase (SOD) and alkyl hydroperoxide reductase (Willmore and Storey, 1997). This enhanced store of antioxidants may be a built-in protection against free radicals (ROS) produced when the brain is reoxygenated after anoxia or even after normal breath-hold dives where arterial oxygen partial pressure (P_{O_2}) can routinely fall to 2.7 kPa (Lutz, 1992).

Surviving anoxia and aerobic recovery

Three distinct phases are involved in fully surviving anoxia: (1) a drastic and immediate downregulation of ATP demand processes, (2) long-term maintenance at basal levels of ATP expenditure and (3) a rapid upregulation when oxygen becomes available.

The first line of defense against anoxic brain failure is a drastic suppression of ATP use during the first 1–2 h of anoxia, lowering energy consumption to such a degree (70–80%) that brain energy needs can be fully met by anaerobic glycolysis (Hochachka and Lutz, 2001). Catastrophe is avoided by a coordinated and tightly regulated downshift in the pathways for ATP demand and supply so that the turtle brain is able to maintain ATP levels and ionic gradients during this dynamic period of energy cost suppression.

The second line of defense involves maintaining, over hours to days, the integrity of the deeply depressed brain at an order of magnitude lower than normoxic levels. In this regard, the brain is much more complicated than other tissues, having to preserve functional activity through a continued interplay between excitation and inhibition and having to defend the intricate architecture of synaptic network connections. As degenerative processes will continue, essential tightly regulated and controlled countermeasures must remain active. Here, the turtle brain is in a minimal or basal state for neuronal survival and can be used to identify those processes fundamental to survival, processes that almost certainly are of wide and general relevance but are not amenable to investigation in the anoxia-intolerant mammalian model.

The third phase is recovery. When O_2 supply is restored, there must be a coordinated and comparatively rapid reactivation of the suppressed neuronal activities in order that the animal can function again for fight, flight and feeding. At this time, the brain must also have in effect defenses against the putative massive generation of destructive reoxygenation-generated ROS. To date, little is known about this aspect of brain recovery, a comparatively neglected area.

To survive, all of these phases must be successfully negotiated, which requires different responses at each step. Responses occur at the molecular, cellular and intercellular levels.

Hypoxia-induced molecular changes

One of the most important hypoxia-driven factors, the hypoxia-inducible HIF-1α, plays a major role in coordinating many adaptive response to hypoxia in the mammal (Haddad, 2002). More than two dozen HIF-1 target genes are known, including genes involved in vascular biology, such as vascular endothelial growth factor (*VEGF*), and genes involved in glucose uptake and glycolysis, such as glucose transporter 1 and phosphofructokinase L (Wenger, 2002). Under normoxic conditions, HIF-1α is hydroxylated by oxygen-dependent propyl hydroxylases and targeted for proteolytic destruction. But, under hypoxic conditions, HIF-1α is stabilized, translocates into the nucleus and activates gene expression (Wenger, 2002). Interestingly, a semi-quantitative RT-PCR analysis found no changes in HIF-1α mRNA during anoxia and subsequent reoxygenation (Prentice et al., 2003), which is consistent with the findings in mammalian systems that HIF-1 regulation does not occur at the transcriptional level but primarily at the post-translational level. That post-translational regulation is also occurring in the turtle is suggested by preliminary observations of DNA binding activity specific to an HIF-1 DNA consensus sequence in the anoxic turtle brain.

It has been widely reported that hypoxia/ischemia produces an increase in the inducible Hsp72 in the mammalian brain, where it is thought to provide protection (Lipton, 1999). In the turtle brain, Hsp72 is also induced in early anoxia, peaking at ~8 h anoxia and then falling to normoxic levels at 12 h anoxia (Prentice et al., in press). However, unlike mammals, where Hsc73 is only slightly inducible, in the turtle brain Hsc73 is progressively elevated over 12 h anoxia (Prentice et al., in press). This differential expression of Hsp proteins suggests that Hsp72 and Hsc73 have different roles during brain anoxia. The (comparatively) short-term rise and fall in Hsp72 indicates that it may have a protective role during the initial transition to the hypometabolic state, a period of substantial metabolic changes. The continued and increased presence of Hsc73 during long-term anoxia suggests that this protein may be involved in 'housekeeping' roles that are necessary to ensure the functional integrity of the neuronal network during the long-term hypometabolic phase.

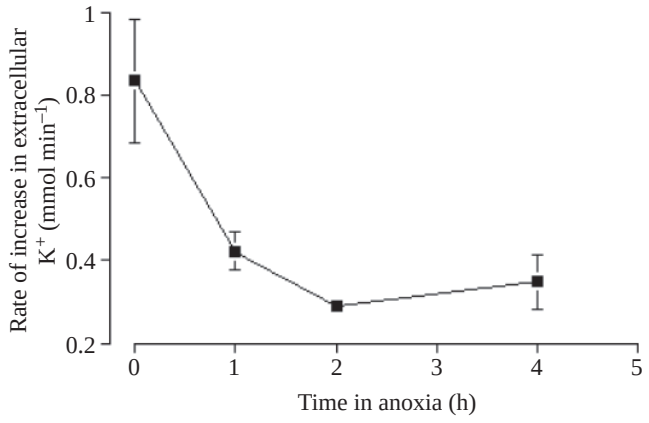


Fig. 1. Progressive decrease in the rate of increase in extracellular K^+ following ouabain superfusion in the anoxic turtle striatum (from Pek and Lutz, 1997).

In mammals, activity levels of NF-κB are increased in both neurons and glia following ischemic stroke and may induce multiple protective genes related to immune function, inflammation, apoptosis and protection against ROS damage (Haddad, 2002; Martindale and Holbrook, 2002). Interestingly, in the anoxic turtle brain, NF-κB shows maximal DNA binding at 6 h of anoxia (Lutz and Prentice, 2002). It is possible that the late translocation of NF-κB to consensus DNA binding sites in the turtle brain is part of a preemptive defense mechanism against reoxygenation ROS damage and incipient apoptosis.

Ion channels

Since ion pumping accounts for more than 50% of the energy requirements of the normoxic neuron, a reduction in ion permeability can have important savings in anoxia, and indeed there is evidence of a decrease in K^+, Na^+ and Ca^{2+} channel activities early in anoxia.

Potassium channels are responsible for setting the resting potential, determining the rate of repolarization and for neuronal firing rates (Meir et al., 1999). And, indeed, potassium flux is reduced by ~50% over the first hour of anoxia and falls to ~35% of normoxic levels by 2 h anoxia, after which no further decline is seen (Fig. 1; Chih et al., 1989; Pek and Lutz, 1998). Activated K_{ATP} channels mediate the downregulation of K^+ efflux during the initial energy crisis period when ATP is depleted (Pek and Lutz, 1998), and adenosine A_1 receptors are also involved in this process (Pek and Lutz, 1998). Voltage-gated K^+ channels (Kv channels) are key determinants of brain electrical activity (Levitan et al., 1998), and several are thought to act as oxygen sensors (Meir et al., 1999). In the mammal, there is evidence that these channels are reversibly blocked by acute hypoxia (Levitan et al., 1998). We have found in the anoxic turtle brain that the gene transcription of Kv1 channels was reduced to 18.5% of normoxic levels (Prentice et al., 2003). At least part of the reduction in K^+ efflux may be related to the downregulation of Kv1 channel protein, as Kv channels are known to have a rapid turnover. A reduction in Kv channel gene expression may be a critical component in the orchestrated reduction in brain energy demand since it would reduce excitability. Kv1 channel mRNA levels were restored following subsequent reoxygenation, indicating that gene transcription of brain Kv channels is reversibly regulated by oxygen supply (Prentice et al., 2003).

The action potential is generated by voltage-gated Na^+ channels (Meir et al., 1999). There is a 42% decrease in the density of voltage-gated Na^+ channels in the anoxic turtle cerebellum (Perez-Pinzon et al., 1992), a probable cause of the corresponding elevation in the action potential threshold (Sick et al., 1993). This would result in a fall in neuronal activity through 'spike' arrest (Sick et al., 1993).

NMDA receptor activity is also progressively reduced during anoxia. Its activity falls by 50–60% over the first

1–8 min anoxia, thereby providing immediate protection against uncontrolled glutamate-activated Ca^{2+} influx (Bickler et al., 2000). This rapid decrease in NMDA receptor activity appears to be controlled by activation of phosphatase 1 or 2A (Bickler and Donohoe, 2002). Adenosine also has a role in promoting NMDA receptor suppression (Buck and Bickler, 1998). As anoxia progresses, the NMDA receptor activity is further depressed but not eliminated (Bickler et al., 2000). This additional decrease in NMDA receptor activity is due to a removal/internalization of receptors from the cell membrane (Bickler et al., 2000).

As there is a substantial downregulation of ion channels during anoxia, one of the first priorities when air breathing is resumed will be to reactivate ion channel functioning. This is indicated by the upregulation of Kv transcription within 4 h of air breathing (Prentice et al., 2003). Potential signals for upregulation include activated O_2 sensors, release of ROS and/or changes in mitochondrial redox status (Prentice et al., 2003).

Neurotransmitters

While a rapid increase in extracellular levels of glutamate and dopamine is a hallmark of hypoxic/ischemic exposure in the mammalian brain, the turtle brain prevents increases in these excitatory neuroactive compounds during anoxia (Nilsson and Lutz, 1991; Milton and Lutz, 1998).

During the first few hours of anoxia, extracellular glutamate levels are maintained by a reduction in glutamate release (mainly due to the inhibition of neuronal vesicular glutamate release), combined with the continued operation of glutamate uptake transporters (Milton et al., 2002). During this period, the reduction in glutamate release is mediated by the activation of adenosine receptors and the opening of K^+_{ATP} channels (Milton et al., 2002). Interestingly, the activation of each pathway appears sufficient to produce the full inhibitory effect, since it is only when both systems are antagonized that the anoxia-induced decrease in glutamate release is prevented (Milton et al., 2002). This indicates a redundancy or back up in control mechanisms during the initial periods of anoxia when ATP levels are low. By contrast, in the energy-deprived mammalian brain, the rapid increase in extracellular glutamate is a result of reduced glutamate uptake combined with increased glutamate release from vesicular and nonvesicular sources (Dawson et al., 2000).

As anoxia progresses, the rate of glutamate release continues to decrease but, in contrast to the initial decrease in glutamate release, this reduction is modulated by adenosine and $GABA_A$ receptors but not K^+_{ATP} channels (Thompson and Lutz, 2001). It thus appears that the signaling/control mechanisms of glutamate release change as the anoxic depression develops, with adenosine and K^+_{ATP} channels being effective during the transition period when ATP is temporarily reduced, and adenosine and GABA being important during extended anoxic exposure after ATP levels have recovered. A similar reduction in the effectiveness of K^+_{ATP} channels to downregulate ion channels in late anoxia has been reported in the anoxic turtle cortex (Pek-Scott and Lutz, 1998).

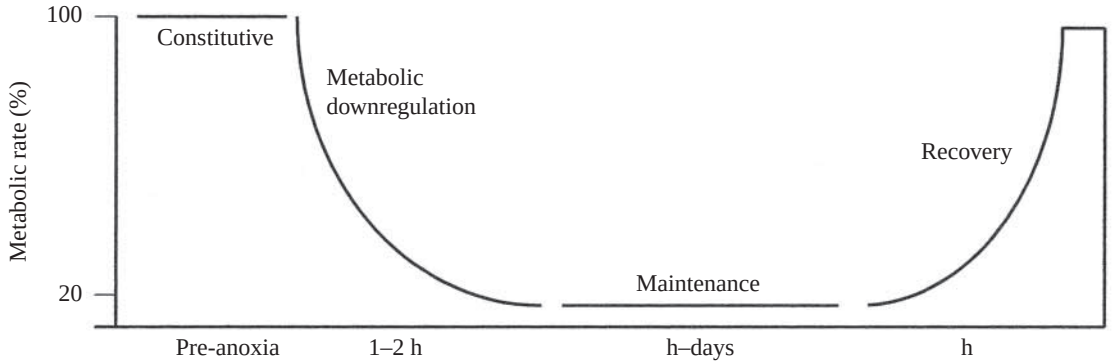
Since the uptake of glutamate is energetically expensive, estimated at 1.5 ATP per glutamate anion (Swanson and Duan, 1999), and the turtle's basic strategy to survive anoxia is to minimize ATP expenditures, the continued transporter activity suggests that glutamate has an important function in anoxia survival. It may be a matter of preserving neuronal integrity since many synaptic connections are dependant on glutamate (Soltesz and Nusser, 2001). If continued glutamate release is essential for the turtle, it is also likely to be so for the mammal, and current efforts to protect against ischemia by pharmacologically blocking the release of glutamate or blocking glutamate receptors may be counterproductive. Indeed, while there have been major efforts to protect against post-ischemic damage by blocking glutamate release, to date no clinically effective glutamate antagonist has been found (Schwartz-Bloom and Sah, 2001). Some have harmful side effects; others are ineffective (Schwartz-Bloom and Sah, 2001).

Low extracellular levels of dopamine (DA) are also maintained in the turtle brain during anoxia through a balance of release and continually active uptake mechanisms. DA homeostasis fails early in the anoxic/ischemic mammalian brain; unlike the widespread release of excitatory amino acids (EAAs), excessive DA release is seen well before high energy stores are fully depleted (Huang et al., 1994). In the hypoxic mammalian brain, increases in extracellular DA are due primarily to decreased reuptake into the cell coupled with increased release from intracellular stores (Huang et al., 1994). By contrast, in the turtle brain during long-term anoxia, DA is continuously released and a balance is maintained by the continued function of reuptake mechanisms (Milton and Lutz, 1998). As with glutamate, this is energetically costly and thus is likely to serve some function in maintaining neuronal circuitry during anoxia.

By contrast to the EAAs, extracellular GABA starts to rise after about 2 h anoxia and continues to increase for at least the next 24 h (Nilsson and Lutz, 1991). Interestingly, there is evidence that Hsc73 is a controlling factor for vesicular accumulation of GABA (Jin et al., 2003), so the increased Hsc73 we have noted in the turtle brain (Prentice et al., in press) may also function in facilitating/controlling GABA release in anoxia.

The rise in GABA is accompanied by an increase in $GABA_A$ receptor number, which continues to increase for at least 24 h (Lutz and Leone-Kabler, 1995). The upregulation in $GABA_A$ receptors may function to increase the effectiveness of the inhibitory action of GABA, strengthening the GABA inhibitory tone during the basal state.

In the ischemic mammalian brain, the dysfunction of GABA neurotransmission is a major contributor towards neuronal death (Schwartz-Bloom and Sah, 2001) and there are current attempts to provide neuroprotection by pharmacologically enhancing GABA neurotransmission. These include preventing GABA reuptake (i.e. enhance levels of extracellular



Constitutive factors	Metabolic downregulation	Basal maintenance	Recovery
Glycogen	Hypoxia/energy failure sensing	ATP turnover rates minimal	Oxygen sensing
Opioid receptors	HIF-1α ↑, glycolysis ↑	Preserve network integrity	Ion channels ↑
GABA receptors	ATP ↓↑, adenosine ↑	Ion channel ↔	Electrical activity ↑
Hsp72, Hsc73	Ion channels ↓, electrical activity ↓	Electrical activity ↔	Protein synthesis ↑
NF-κB	Glutamate, dopamine release ↓	Glutamate release ↔	
PACAP 38	Protein synthesis ↓	Dopamine release ↔	ROS defenses ↑
Ascorbate	Hsp72 ↑, Hsc73 ↑	GABA release ↑	
SOD		GABA receptors ↑	
	Suppression of ATP turnover rates	Protein synthesis ↔	
		Hsp72 ↓, Hsc73 ↑	

Fig. 2. A diagrammatic overview of some of the factors involved in negotiating anoxia survival in the turtle brain. Constitutive factors that predispose for anoxia tolerance include enhanced levels of glycogen stores, elevated heat shock protein, increased densities of protective receptors and elevated antioxidant capacities. Three distinct phases are involved in surviving and recovering from anoxia. (1) A coordinated downregulation of ATP demand processes to ~20% of normoxic levels. This phase, which takes 1–2 h, includes a reduction in ion channel and electrical activities, a reduction in glutamate and dopamine release, a reduction in protein synthesis and a substantial increase in Hsp72 and Hsc73 levels. (2) Long-term survival (days) at basal levels of ATP expenditure. Neuronal network integrity is preserved through the continued operation of core activities. These include periodic electrical activity, an increased release of GABA and a reduced but continued release of glutamate and dopamine. There is a further increase in Hsc73, indicating a 'housekeeping' role for this protein during this period. (3) When oxygen becomes available there is a rapid upregulation of neuronal processes to restore full function, together with the activation of protection mechanisms against reperfusion-generated reactive oxygen species (ROS).

GABA) and increasing $GABA_A$ receptor activity with agonists (Schwartz-Bloom and Sah, 2001). As both strategies are used by the turtle, the turtle may provide useful lessons for effective GABAergic-related strategies.

Electrical activity

Important energy savings come from an almost full suppression in turtle brain electrical activity during anoxia, but this comes about in a complex manner (Fernandes et al., 1997). During the first 100 min of N_2 respiration, electroencephalogram (EEG) amplitude is progressively reduced, with low-amplitude slow-wave activity predominating (3–12 Hz), and the total EEG power spectra decreased across all frequencies to about one order of magnitude lower than during normoxia. Most interestingly, during this period, 3 s bursts of high-voltage (24 μV), slow, rhythmic waves (3–8 Hz) appeared, similar to the theta waves associated in mammals and birds with slow-wave sleep. This synchronization of brain electrical activity may relate to a coordinated down-switching of brain electrical activities.

During the subsequent anoxic basal state, the electrical activity is greatly reduced, the EEG amplitude is ~20% of the normoxic level and the total EEG power is an order of magnitude lower. Corresponding to the continued low level of electrical activity, ion pumps, although depressed, are still active (Sick et al., 1993). However, this depressed activity state is periodically (0.5–2 min^{-1}) interrupted by short bursts (2–15 s) of mixed frequency activity. The burst activity may be necessary to maintain circuit integrity or may be part of a periodic check for a signal for arousal. The continued release and uptake of neurotransmitters such as glutamate (Milton et al., 2002) and dopamine (Milton and Lutz, 1998) may be determinant factors for the sustained periodic burst activity in the anoxic turtle brain.

There is a full recovery of the EEG (Fernandes et al., 1997) and evoked potential amplitudes (Feng et al., 1990) within 2 h of reoxygenation.

ROS

While transient fluctuations in ROS serve important regulatory functions, high and sustained levels can cause severe damage to DNA, protein and lipids and cause apoptosis and necrosis (Martindale and Holbrook, 2002). For the turtle, free radicals pose a special problem during the recovery period.

A few minutes of anoxia are sufficient to reduce the mitochondrial respiratory chain electron carriers to such an extent that toxic amounts of ROS will be generated when oxygen supply is restored. Clearly, the turtle brain is in a prime condition to experience massive amounts of ROS when it is reoxygenated after many hours of anoxia. That the turtle brain survives this insult is indicated by studies of neuronal cell culture, which are unaffected by exposure to 2 days anoxia and 1 day reoxygenation, conditions that would be fatal for mammalian neurones (Lutz et al., 2003b). While it is possible that the turtle has a means to prevent ROS formation, it is more likely that it has mechanisms to protect against ROS damage. Evidence of high constitutive antioxidant protection in the normoxic animal was discussed above and it is also possible that there is an upregulation of antioxidant capacities during aerobic recovery. The translocation of NF-κB in late anoxia could mediate the activation of antioxidant genes, since there is evidence that, in the mammalian brain, NF-κB mediates post-ischemic free radical damage (Lipton, 1999). The turtle may also have enhanced defenses against oxidative damage. For example, in contrast to mammals, there is little evidence in the turtle of lipid peroxidation damage during anoxia or recovery (Willmore and Storey, 1997).

Conclusions

Recent research makes it abundantly clear that the turtle brain's capacity to survive and recover from anoxia is not simply one of passive tolerance, but is due to a complex interlocking cluster of adaptations to produce and maintain a state of deep hypometabolism and to negotiate a successful revival on reoxygenation. The constitutive and expressed factors behind the turtle brain's extraordinary ability to tolerate anoxia are illustrated in Fig. 2. Constitutive factors that predispose for anoxia tolerance include enhanced levels of glycogen stores, increased densities of protective receptors, elevated antioxidant capacities and elevated heat shock protein. However, to survive an anoxic insult, three distinct phases must be negotiated successfully. (1) A coordinated downregulation of ATP demand processes to basal levels. This phase, which takes 1–2 h, includes a reduction in Kv channel transcription and a substantial increase in Hsp72 and Hsc73 levels. Adenosine and K_{ATP} channels mediate in several key events, including the initiation of channel arrest and a reduction in the release of EAAs. (2) Long-term survival (days) at basal levels of ATP expenditure. Neuronal network integrity is preserved through the continued operation of core activities. These include periodic electrical activity, an increased release of GABA and a continued release of glutamate and dopamine. Adenosine and GABA modulate glutamate release. There is a further increase in Hsc73, indicating a 'housekeeping' role for this protein during this period. Protein synthesis, although substantially reduced (Fraser et al., 2001), is maintained at basal levels compatible with neuronal network survival (Lutz et al., 2003a). (3) A rapid upregulation of neuronal processes when oxygen becomes available to restore full function, together with protection against reperfusion-generated ROS. In general, study of this post-anoxic recovery phase has been neglected, but it has great potential to enhance our understanding of mammalian ischemic-reperfusion survival.

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