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Slow ATP loss and the defense of ion homeostasis in the anoxic frog brain

Debra L. Knickerbocker and Peter L. Lutz*

Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA

*Author for correspondence (e-mail: lutz@fau.edu)

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Summary

For most vertebrates, cutting off the oxygen supply to the brain results in a rapid (within minutes) loss of ATP, the failure of ATP-dependent ion-transport process, subsequent anoxic depolarization of neuronal membrane potential and consequential neuronal death. The few species that survive brain anoxia for days or months, such as the freshwater turtle *Trachemys scripta*, avoid anoxic depolarization and maintain brain ATP levels through a coordinated downregulation of brain energy demand processes. The frog *Rana pipiens* represents an intermediate in anoxia-tolerance, being able to survive brain anoxia for hours. However, the anoxic frog brain does not defend its energy stores. Instead, anoxia-tolerance appears to be related to a retarded rate of ATP depletion. To investigate the relationship between this slow ATP depletion and the loss of ionic homeostasis, cerebral extracellular K^+ concentrations were monitored and ATP levels measured during anoxia, during the initial phase of anoxic depolarization and during complete anoxic depolarization. Extracellular K^+ levels were maintained at normoxic levels for at least 3 h of anoxia, while ATP content decreased by 35 %. When ATP levels reached 0.33±0.06 mmol l^{-1} (mean ± S.E.M., N=5), extracellular K^+ levels slowly started to increase. This value is thought to represent a critical ATP concentration for the maintenance of ion homeostasis. When extracellular [K^+] reached an inflection value of 4.77±0.84 mmol l^{-1} (mean ± S.E.M., N=5), approximately 1 h later, the brain quickly depolarized. Part of the reduction in ATP demand was attributable to an approximately 50 % decrease in the rate of K^+ efflux from the anoxic frog brain, which would also contribute to the retarded rate of increase in extracellular [K^+] during the initial phase of anoxic depolarization. However, unlike the anoxia-tolerant turtle brain, adenosine did not appear to be involved in the downregulation of K^+ leakage in the frog brain. The increased anoxia-tolerance of the frog brain is thought to be a matter more of slow death than of enhanced protective mechanisms.

Key words: brain anoxia, adenosine, ATP, frog, *Rana pipiens*, channel arrest, depolarization.

Introduction

There is a vast literature on the effects of anoxia/ischemia on the mammalian brain [for a review, see (Lipton, 1999)]. In essence, unable to compromise on its intense energy consumption, the mammalian brain loses ATP within minutes of being deprived of oxygen. There follows a consequent failure of ATP-dependent ion exchangers, 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 and Nilsson, 1997).

There are, however, a few truly anoxia-tolerant exceptions, such as the freshwater turtle *Trachemys scripta* and the crucian carp *Carassius carassius*, which have brains that are able to survive days of anoxia at room temperature (Lutz and Nilsson, 1997). The most important compensation in the turtle brain for surviving anoxia is, almost uniquely, lowering its energy consumption to such a degree (by 70–80 %) that brain energy needs can be fully met by anaerobic glycolysis (Lutz et al., 1984; Doll et al., 1993). As a result, the turtle brain is able to maintain ATP levels and ionic gradients during anoxia and thus avoid the fatal consequences of energy failure (Lutz, 1992). A reduction in membrane ion leakage (channel arrest) may provide important energy savings for the anoxia-tolerant brain (Lutz et al., 1985; Hochachka, 1986; Hand and Hardewig, 1996), and there is evidence that the rate of K^+ flux is significantly lower in the anoxic turtle brain than in the normoxic brain (Chih et al., 1989; Jiang and Haddad, 1991; Pek and Lutz, 1997).

Some frog species (*Rana temporaria*, *Rana pipiens*) appear to be intermediate in anoxia-tolerance, having brains that can tolerate approximately 3 h without oxygen at room temperature (Lutz and Reiners, 1997; Wegener and Krause, 1993) and at least 30 h of anoxia at 5 °C (Hermes-Lima and Storey, 1996). The peculiarity is that, unlike anoxia-tolerant species, these

frogs do not defend brain ATP levels during anoxia but, in contrast to anoxia-vulnerable species, ATP depletion takes hours instead of minutes (Okada and McDougal, 1971; Lutz and Reiners, 1997; Wegener and Krause, 1993). After approximately 100 min of anoxia, there is a release of adenosine, but an excitotoxic release of neurotransmitters does not occur until after 3 h of anoxia (Lutz and Reiners, 1997). However, it is not known whether ionic gradients are maintained during the hours of anoxia survival in the frog brain, a critical factor in understanding this phenomenon.

The release of adenosine may be a factor in anoxia-tolerance in the frog brain (Lutz and Reiners, 1997). Extracellular adenosine plays an important protective role in the turtle (Lutz and Nilsson, 1997) and mammalian (Sweeney, 1996) brain and appears to be involved in the activation of channel arrest at the onset of anoxia in the turtle brain (Pék-Scott and Lutz, 1998). Whether it has such a role in the frog brain is not known.

The frog brain therefore offers a unique model in which to examine the relationship between ATP depletion and the failure of ionic-gradient-maintaining pumps. It is also of interest to investigate the mechanisms that slow ATP loss in the anoxic frog brain.

The aims of this study were (i) to determine whether extracellular [K^+] is maintained in the anoxic frog brain over the known period of survival, (ii) to identify a critical ATP level at which ionic homeostasis is lost, (iii) to determine whether a decrease in the rate of K^+ efflux is an energy-saving strategy in the anoxic frog brain and (iv) to investigate whether adenosine plays a role in anoxia survival in the frog brain.

Materials and methods

Studies were conducted using *Rana pipiens*, weighing 30–55 g, collected by commercial suppliers (Charles D. Sullivan Co. Inc., Nashville, TN, USA). The animals were housed in plastic pens at 25 °C and had constant access to fresh water; they were fed with crickets and kept on a 12 h:12 h light:dark cycle. Experimental procedures were approved by the FAU Institutional Animal Care and Use Committee and all applicable NIH guidelines were maintained.

Animal preparation

The frogs were placed in a 500 ml sealed polyethylene box containing saline in which was dissolved the anesthetic tricaine methanesulfonate, MS222 (250–300 g l^{-1}), buffered to pH 7.4. Until surgery, this chamber was continuously aerated with 100 % O_2 to ensure that the anesthetized frogs remained fully aerobic. After a surgical plane had been achieved (as indicated by the lack of an ocular reflex), an area of the skull (4 mm×5 mm) directly over the optic lobes was thinned and removed with jeweller's forceps, exposing the optic tectum. In all experiments, the brain was superfused with frog ACSF (116 mmol l^{-1} NaCl, 2 mmol l^{-1} KCl, 26 mmol l^{-1} $NaHCO_3$, 1.25 mmol l^{-1} NaH_2PO_4, 2 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgSO_4$, 2 mmol l^{-1} glucose, buffered to pH 7.4) at a flow rate of approximately 1 ml h^{-1}. The chamber was sealed with a tight-fitting lid during the entire experiment, except for a small hole above the brain for the placement of the microelectrode. The water temperature in the sealed aerated chamber was 16 °C. Anoxic conditions were achieved by aerating the chamber with 100 % N_2 (County Welding/Air Products).

Using a micromanipulator, a double-barrelled K^+-selective glass microelectrode (Sick and Kreisman, 1981) was inserted 500–700 μm below the dorsal surface of the optic lobe. Calibration of electrical activities with K^+ was performed before and after experimentation using different concentrations of KCl, with NaCl maintaining a constant ionic strength of 200 mosmol l^{-1}. Electrodes were used only if they showed stability and sensitivity to changing K^+ concentrations (a voltage reading change of at least 70 mV in response to a change from a 1 mmol l^{-1} to a 50 mmol l^{-1} solution) during the calibration.

Experimental groups

To investigate the levels of extracellular K^+ during anoxia and their relationship to brain ATP levels, experiments were performed on four groups of five frogs. Brain extracellular [K^+] was monitored in all frogs, and at the end of each experiment the brain was dissected out and frozen in liquid nitrogen in less than 1 min. Tissues were then stored at −80 °C until used.

In group A (normoxic control), individual animals were aerated with O_2 for 30 min. Group B frogs were aerated with N_2 for 1 h. Only animals that showed no increase in extracellular [K^+] from either group were used. Group C animals were left in anoxic conditions until extracellular [K^+] was seen to increase (phase 1 of anoxic depolarization) (Hansen, 1977). Group D animals were left in anoxic conditions until complete anoxic depolarization (see Fig. 1).

The whole brains were processed and analyzed for ATP content (Nilsson and Lutz, 1991) using high-performance liquid chromatography (HPLC) on an Absorbosphere C18 column (Alltech) connected to an ultraviolet detector (Nilsson and Lutz, 1991).

To investigate the effects of anoxia on K^+ efflux, the frogs were allocated to four groups. At fixed points, 10 mmol l^{-1} ouabain was superfused onto the brain to inhibit Na^+/K^+-ATPase (Chih et al., 1989; Pék-Scott and Lutz, 1998) and in some experiments theophylline was added to inhibit adenosine receptors (Pék and Lutz, 1997). Group 1 was normoxic for 45 min before ouabain was added. Group 2 was held in anoxia for 2 h, and the brain was then superfused with ouabain. In normoxic group 3, the brain was superfused with ACSF containing 100 μmol l^{-1} theophylline for 1 h before ouabain was added to ensure sufficient blocking of adenosine receptors (Pék and Lutz, 1997). A similar procedure (theophylline then ouabain) was adopted for the fourth group after 2 h of N_2 aeration.

All statistical analyses were performed using analysis of variance (ANOVA) and Dunnett's tests. All data are expressed as means ± S.E.M.

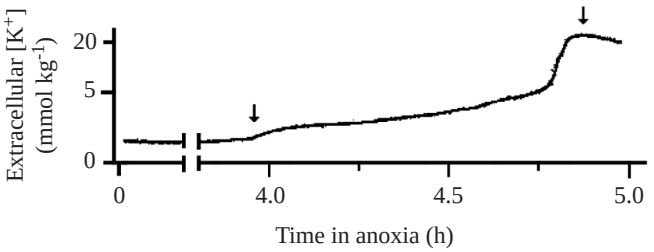

Fig. 1. Typical changes in extracellular [K^+] in a frog brain during 5 h of anoxia. While monitoring [K^+], brain samples were frozen during normoxia, at 1 h of anoxia and at the marked events (arrows) for measurement of ATP levels.

Results

In these experiments, the normoxic extracellular K^+ level in the frog optic tectum was 1.52±0.12 mmol l^{-1} (*N*=5), identical to that found by Sick and Kreisman (Sick and Kreisman, 1981). This value was maintained for the first 3 h of anoxia (185.9±12.6 min, *N*=5, Fig. 1), then it slowly drifted up. By 4 h of anoxia, the extracellular [K^+] had increased significantly over normoxic levels ($P<0.05$), and it reached a plateau after approximately 5 h of anoxia (284±8.94 min). The peak level of extracellular [K^+] reached after anoxic depolarization was 16.85±2.55 mmol l^{-1}.

The normoxic ATP concentration in frog brain was 0.95±0.06 mmol kg^{-1}, at the low end of the range previously measured for this species (1.1–1.56 mmol kg^{-1}) (McDougal et al., 1968). Brain ATP had fallen within the first hour and was almost fully depleted in 4 h (Fig. 2). Previous researcher have similarly found that frog brain ATP levels are not defended during anoxia (Wegener and Krause, 1993; Lutz and Reiners, 1997).

To link brain energy status and loss of ion homeostasis during anoxia, whole-brain ATP content was measured at the points when extracellular K^+ levels started to rise and when extracellular [K^+] plateaued (Fig. 3). The 'critical' ATP level, at the beginning of phase 1 for K^+ efflux, averaged 0.33±0.06 mmol kg^{-1} (*N*=5), which was 35 % of control values. The ATP level at anoxic depolarization was 0.042±0.011 mmol kg^{-1}.

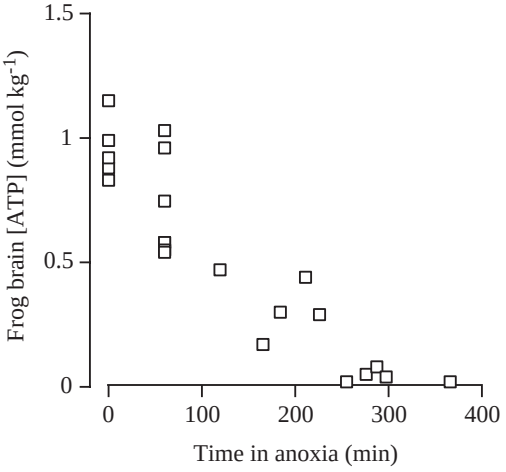

Fig. 2. Changes in frog brain ATP levels during anoxia. ATP levels are not defended.

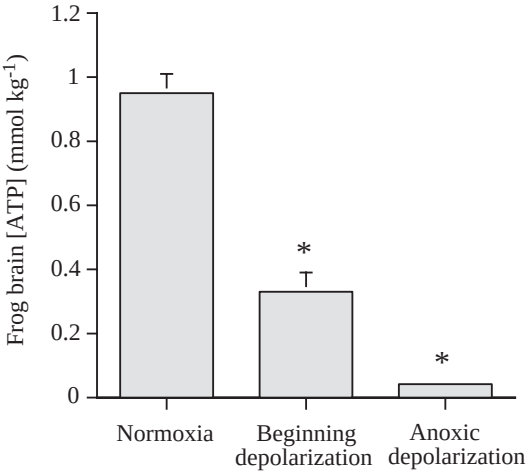

Fig. 3. Brain ATP levels during normoxia and during anoxia when extracellular [K^+] started to increase (beginning of depolarization) and when anoxic depolarization had been reached. Values are means + S.E.M., *N*=5. *Statistically different from the normoxic value ($P<0.05$).

Fig. 4 shows typical changes in extracellular [K^+] in brains following superfusion with 10 mmol l^{-1} ouabain in frogs breathing air and after 2 h of N_2 respiration. In all cases, the pattern of change in extracellular [K^+] was similar to that described for the anoxic mammalian brain (Hansen, 1977), i.e. a slow increase in [K^+] (phase 1) to a threshold or inflection value, followed by a rapid increase (phase 2) to a plateau associated with anoxic depolarization (phase 3).

K^+ leakage rate was calculated by measuring changes in extracellular [K^+] during ouabain superfusion. The rate of K^+ leakage in phase 1 is thought to best represent resting K^+ leakage in the brain because K^+ conductance may be affected by other factors when the depolarization produced by increasing extracellular [K^+] is sufficiently large to open voltage-gated channels (phase 2) (Chih et al., 1989; Pék-Scott

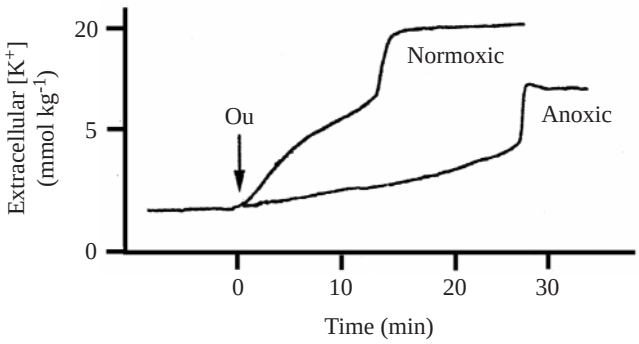

Fig. 4. Traces showing changes in extracellular [K^+] in frog brain after administration of ouabain (Ou, arrow) during air respiration (normoxic) and after 2 h of 100 % N_2 respiration (anoxic).

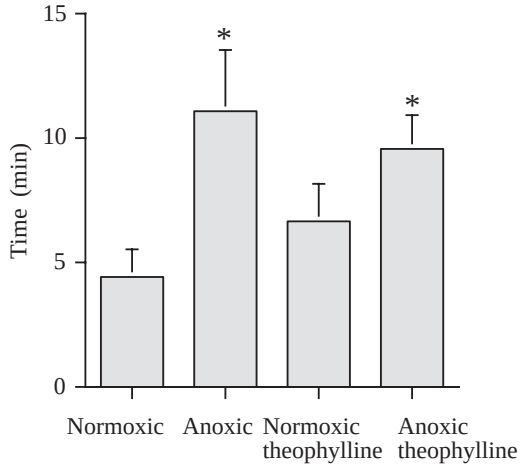

Fig. 5. Time taken to increase extracellular [K^+] by 1 mmol l^{-1} above basal values after superfusion with ouabain during normoxia, after 2 h of anoxia, during normoxia in the presence of theophylline and after 2 h of anoxia in the presence of theophylline. Values are means + S.E.M., *N*=5. *Statistically different from the value for the corresponding normoxic group (*P*<0.05).

and Lutz, 1998). An estimate of the rate of K^+ leakage during phase 1 was obtained by measuring the time it took extracellular [K^+] to increase by 1 mmol l^{-1} over baseline levels following ouabain application. The time to reach maximal extracellular [K^+], which would correspond to full depolarization, was also measured.

Compared with air-breathing animals, the time taken for extracellular [K^+] to increase by 1 mmol l^{-1} over resting levels had more than doubled after 2 h of anoxia (+251 %), indicating a corresponding reduction in the rate of K^+ leakage (Fig. 5). However, the value of the inflection point, after which [K^+] increased rapidly, did not change (4.80±0.87 mmol l^{-1} in normoxia, 4.77±0.84 mmol l^{-1} in anoxia). As a consequence, the time to reach the critical extracellular [K^+], i.e. the duration of phase 1, also increased in anoxia (normoxic 11.9±2.1 min *versus* anoxic 21.5±1.8 min, *P*<0.05). During phase 2, [K^+] increased rapidly in both groups, and there was no significant difference in the total duration of phase 2 between normoxic (2.84±0.39 min) and anoxic (2.24±0.33 min) animals.

Blocking adenosine receptors with theophylline had no measurable effect on the rates of K^+ leakage in either normoxic or anoxic brains (Fig. 5), indicating that, unlike in the turtle (Pék and Lutz, 1997), adenosine-mediated processes might not be involved in the anoxia-associated reduction in K^+ efflux.

Discussion

This study agrees with earlier studies showing that frogs (*R. pipiens* and *R. temporaria*) do not defend brain ATP levels during anoxia (Wegener and Krause, 1993; Lutz and Reiner, 1997). The 2.5-fold more rapid fall in ATP levels measured in an earlier study is in large part due to the differences in experimental temperatures (16 °C in the present study, 25 °C in Lutz and Reiner, 1997). The collapse in ATP levels is similar to the more rapid response seen in anoxia-intolerant mammals (Siesjö, 1978) and fish (van Raaij et al., 1994), but it is quite unlike that shown by long-term anoxic survivors, such as the turtle *T. scripta* and the crucian carp, which maintain brain ATP levels for many hours of anoxia (Lutz and Nilsson, 1997). However, while the loss of ATP results in rapid (within minutes) membrane depolarization in the mammalian brain, with consequent degenerative changes, the frog maintained extracellular K^+ levels for over 3 h of anoxia before levels slowly started to drift up. After a further approximately 1.5 h, a threshold value was reached ([K^+]=4.77±0.84 mmol l^{-1}) which, as in mammals, was followed by a rapid increase in extracellular [K^+] to a plateau indicative of anoxic depolarization. The time to anoxic depolarization corresponds to the time of the massive loss of neurotransmitters (GABA, glutamate) from the anoxic frog brain (Lutz and Reiners, 1997). In mammals and the turtle, both GABA and neurotoxic amounts of glutamate are rapidly released during anoxic depolarization (Lutz and Nilsson, 1997).

Simultaneous measurements of the relatively slow changes in K^+ and ATP levels in the anoxic turtle brain allowed a determination of the critical ATP levels at which ionic homeostasis starts to be lost. In the rat, there is a precipitous efflux of K^+ when ATP levels fall below 50 % of normoxic values (Erecinska and Silver, 1998; Katsura et al., 1994), and this is followed by a rapid increase in extracellular glutamate and GABA levels as ATP continues to be depleted (Shimizu et al., 1993). But, in the mammal, the precise critical level of ATP at which the uncontrolled efflux of K^+ occurs is difficult to determine because the anoxia-driven changes are so rapid and because pathological events and defense mechanisms are inextricably mixed. The ‘critical ATP level’ determined in the present study was 0.33±0.06 mmol l^{-1}, which is 35 % of normoxic levels. These values are thought to represent an energy minimum for ion homeostasis to be maintained. In the mammal, the opening of K_{ATP} channels following ATP depletion is thought to be a major route of K^+ efflux (Jiang et al., 1992).

The 50 % reduction in the rate of K^+ leakage in anoxic the frog brain is one factor contributing to the delayed time to the onset of depolarization. It would lower the cost of ion pumping (and consequently the rate of depletion of ATP) and the rate of increase in extracellular [K^+] (phase 1) (and consequently extend the time to reach the extracellular [K^+] threshold value). A similar but greater reduction (70 %) in the rate of K^+ efflux is seen in the anoxic turtle brain (Pék and Lutz, 1997). Interestingly, in sharp contrast to the brain, the frog *R. esculanta* defends skeletal muscle ATP levels during anoxia (Wegener and Krause, 1993) and there is a 75 % decrease in the rate of K^+ leakage in severely hypoxic muscle (Donohoe et al., 2000).

Although adenosine is released during anoxia in the frog brain, unlike in the turtle, it did not appear to play a role in slowing the K^+ efflux (Pék and Lutz, 1997). In the turtle and rat, adenosine is released early in the energy crisis and acts as

a retaliatory metabolite to restore energy balance (Sweeney, 1996; Lutz and Nilsson, 1997), but in the frog adenosine is not released until after ATP has been almost fully depleted (Lutz and Reiners, 1997). The late release of adenosine in the frog may be a pathological event associated with energy failure. It is possible that, in the frog, the reduction in the rate of K^+ efflux is a consequence of the suppression of action potentials and synaptic transmission, 'spike arrest' (Sick et al., 1993), rather than 'channel arrest'. Transmembrane ionic flux would be lower in a quiescent neuron. Indeed, there is evidence of a significant reduction in neuronal activity in the frog brain during early anoxia: in the frog optic tectum, signal transmission disappeared within 30 min of anoxia (Wegener and Krause, 1993) and there was a complete (but reversible) suppression of action potentials in the isolated sciatic nerve within 60 min of anoxia (Okada et al., 1971).

In conclusion, the enhanced anoxia-tolerance of the frog brain is due to a delay in reaching the depolarizing threshold value of extracellular $[K^+]$, i.e. phase 1 is extended. This is achieved in part by reducing the rate of K^+ leakage, which lessens the costs of ion pumping, diminishes the rate of decline of ATP levels and slows the rate of increase in extracellular $[K^+]$. However, when the threshold value of extracellular $[K^+]$ is reached, rapid depolarization follows, with the loss of excitotoxic amino acids and consequent neuronal death. The anoxic frog brain therefore experiences the same sequence of degenerative changes as the mammalian brain but on a greatly extended time scale. The anoxic frog brain dies very slowly

References

Chih, C., Rosenthal, M. and Sick, T. J. (1989). Ion leakage is reduced during anoxia in turtle brain: a potential survival strategy. *Am. J. Physiol.* **257**, R1562–R1564.

Doll, C. J., Hochachka, P. W. and Reiner, P. B. (1993). Reduced ionic conductance in turtle brain. *Am. J. Physiol.* **265**, R929–R933.

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

Erecinska, M. and Silver, I. A. (1989). ATP and brain function. *J. Cereb. Blood Flow Metab.* **9**, 2–19.

Hand, S. C. and Hardewig, I. (1996). Downregulation of cellular metabolism during environmenal stress: mechanisms and implications *Annu. Rev. Physiol.* **58**, 539–563.

Hansen, A. J. (1977). Extracellular potassium concentration in juvenile and adult rat brain cortex during anoxia. *Acta Physiol. Scand.* **99**, 412–420.

Hermes-Lima, M. and Storey, K. B. (1996). Relationship between anoxia exposure and antioxidant status in the frog *Rana pipiens*. *Am. J. Physiol.* **271**, 991–996.

Hochachka, P. W. (1986). Defense strategies against hypoxia and hypothermia. *Science* **31**, 234–241.

Jiang, C. and Haddad, G. G. (1991). Effect of anoxia on intracellular and extracellular potassium activity in hypoglossal neurons *in vitro*. *J. Neurophysiol.* **66**, 103–111.

Jiang, C., Xia, Y. and Haddad, G. G. (1992). Role of ATP-sensitive K^+ channels during anoxia: major differences between rat (newborn and adult) and turtle neurones. *J. Physiol., Lond.* **448**, 599–612.

Katsura, K., Minimisawa, H., Eckholm, B., Folbergrova, J. and Siesjö, B. K. (1994). Changes of labile metabolites during anoxia in moderately hypo- and hyperthermic rats: correlations to membrane fluxes of K^+. *Brain Res.* **590**, 6–12.

Lipton, P. (1999). Ischemic cell death in neurones. *Physiol. Rev.* **79**, 1432–1568.

Lutz, P. L. (1992). Anoxic defense mechanisms in the vertebrate brain. *Annu. Rev. Physiol.* **54**, 601–618.

Lutz, P. L., McMahon, P., Rosenthal, M. and Sick, T. J. (1984). Relationship between aerobic and anaerobic energy production in turtle brain in situ. *Am. J. Physiol.* **247**, R740–R744.

Lutz, P. L. and Nilsson, G. E. (1997). *The Brain Without Oxygen: Causes of Failure and Mechanisms for Survival*. Second edition. Town: Chapman & Hall; Springer-Verlag.

Lutz, P. L. and Reiners, R. (1997). Survival of energy failure in the anoxic frog brain: delayed release of glutamate. *J. Exp. Biol.* **200**, 2913–2917.

Lutz, P. L., Rosenthal, M. and Sick, T. J. (1985). Living without oxygen: turtle brain as a model of anaerobic metabolism. *Molec. Physiol.* **8**, 411–425.

McDougal, D. B., Holowach, J., Howe, M. C., Jones, E. M. and Thomas, C. A. (1968). The effects of anoxia upon energy sources and selected metabolic intermediates in the brains of fish, frog and turtle. *J. Neurochem.* **15**, 577–588.

Nilsson, G. E. and Lutz, P. L. (1991). Release of inhibitory neurotransmitters in response to anoxia in the turtle brain. *Am. J. Physiol.* **261**, R32–R37.

Okada, Y. and McDougal, D. B. (1971). Physiological and biochemical changes in frog sciatic nerve during anoxia and recovery. *J. Neurochem.* **18**, 2335–2353.

Pék, M and Lutz, P. L. (1997). Role for adenosine in channel arrest in the anoxic turtle brain. *J. Exp. Biol.* **200**, 1913–1917.

Pék-Scott, M. and Lutz, P. L. (1998). ATP-sensitive K^+ channel activation provides transient protection to the anoxic turtle brain. *Am. J. Physiol.* **275**, R2023–R2027.

Shimuzu, H., Graham, S. H., Chang, L. L., Mintorovitch, J., James, T. L., Faden, A. I. and Weinstein, P. R. (1993). Relationship between extracellular neurotransmitter amino acids and energy metabolism during cerebral ischemia in rats monitored by microdialysis and *in vivo* magnetic resonance spectroscopy. *Brain Res.* **603**, 33–43.

Sick, T. J. and Kreisman, N. R. (1981). Potassium ion homeostasis in amphibian brain: contribution of active transport and oxidative metabolism. *J. Neurophysiol.* **45**, 998–1012.

Sick, T. J., Pérez-Pinzón, M., Lutz, P. L. and Rosenthal, M. (1993). Maintaining a coupled metabolism and membrane function in anoxic brain: a comparison between turtle and the rat. In *Surviving Hypoxia* (ed. P. W. Hochachka, P. L. Lutz, T. J. Sick, M. Rosenthal and G. van den Thillart), pp. 351–364. Boca Raton, FL: CRC Press.

Siesjö, B. K. (1978). *Brain Energy Metabolism*. Chichester: Wiley.

Sweeney, M. I. (1997). Neuroprotective effects of adenosine in cerebral ischemia: window of opportunity. *Neurosci. Biobehav. Rev.* **21**, 207–217.

van Raaij, M. T. M., Bakker, E., Nieveen, M. C., Zirkzee, H. and van den Thillart, G. E. E. J. M. (1994). Energy status and free fatty acid patterns in tissues of common carp (*Cyprinus carpio*, L.) and rainbow trout (*Oncorhynchus mykiss*, L.) during severe oxygen restriction. *Comp. Biochem. Physiol.* **109A**, 755–767.

Wegener, G. and Krause, U. (1993). Environmental and exercise anaerobiosis in frogs. In *Surviving Hypoxia* (ed. P. W. Hochachka, P. L. Lutz, T. J. Sick, M. Rosenthal and G. van den Thillart), pp. 217–236. Boca Raton, FL: CRC Press.