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CONTRASTING STRATEGIES FOR ANOXIC BRAIN SURVIVAL – GLYCOLYSIS UP OR DOWN

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Summary

Anoxia-tolerant turtles and carp (*Carassius*) exhibit contrasting strategies for anoxic brain survival. In the turtle brain, the energy consumption is deeply depressed to the extent of producing a comatose-like state. Brain metabolic depression is brought about by activating channel arrest to reduce ion flux and through the release of inhibitory γ-aminobutyric acid (GABA) and the up-regulation of $GABA_A$ receptors. Key glycolytic enzymes are down-regulated during prolonged anoxia. The result is a suppression of neurotransmission and a substantial depression in brain electrical activity.

By contrast, *Carassius* remain active during anoxia, though at a reduced level. As in the turtle, there is an adenosine-mediated increase in brain blood flow but, in contrast to the turtle, this increase is sustained throughout the anoxic period. Key glycolytic enzymes are up-regulated and anaerobic glycolysis is enhanced. There is no evidence of channel arrest in *Carassius* brain. The probable result is that electrical activity in the brain is not suppressed but instead maintained at a level sufficient to regulate and control the locomotory and sensory activities of the anoxic carp.

The key adaptations permitting the continued high level of glycolysis in *Carassius* are the production and excretion of ethanol as the glycolytic end-product, which avoids self-pollution by lactate produced during glycolysis that occurs in other vertebrates.

Key words: anoxia, glycolysis, lactate, brain, ethanol adenosine, *Carassius* sp., *Chrysemys* sp., *Trachemys* sp., carp, goldfish, turtle.

Introduction

When anoxia-tolerant animals are faced with a serious deprivation of oxygen, two contrasting responses can be called upon. One is to shut down metabolic operations as much a possible and to go into a holding ‘suspended animation’ mode until oxygen supplies return. The other is to remain active, but at a reduced rate fuelled by non-oxidative energy-producing pathways. For the brain of these animals, the demands of these two strategies present quite different challenges. Both must employ mechanisms for the brain to survive anoxia, but whereas the former strategy causes the brain to shut down to a comatose-like state, the latter requires the brain to continue to function. The brain of the active anoxic animal must still coordinate locomotion and receive and process sensory information. This difference in requirements suggests that there will be no single theory for anoxic brain survival in vertebrates. The mechanisms employed will depend upon the functions to be performed.

Such divergent responses are particularly well illustrated in comparisons of the best-described anoxia-tolerant vertebrates, the fishes of the genus *Carassius* (the crucian carp *C. carassius* and goldfish *C. auratus*) and freshwater turtles of the genera *Trachemys* and *Chrysemys*. These animals are able to survive months of anoxia at low temperatures and days at room temperature. The anoxic turtle is completely inactive, limp, unmoving and unresponsive to external stimuli. The anoxic crucian carp, by contrast, continues to swim, though at a reduced rate (Nilsson *et al.* 1993). Indeed, anoxic crucian carp still respond to external stimuli and are able to avoid attempts by the experimenter to capture them (G. E. Nilsson, personal observations). Since there are many thousands of anoxia-intolerant species between these two and a common ancestor, *Carassius* and turtles have obviously evolved their abilities to survive anoxia separately.

A fundamental distinction between these two responses is seen in the employment of glycolysis, the only energy source during anoxia. Since glycolysis yields less than 10 % of the ATP produced by aerobic metabolism, there are only two possible ways of maintaining ATP levels in the absence of oxygen: the first being to increase the rate of glycolysis – the *glycolyic strategy* – and the second being to depress the rate of ATP use – the *metabolic depression strategy*.

There are clear trade-offs with both strategies. In up-regulating glycolysis, glycogen stores become limiting, and these stores will have to be substantial for any long-term anoxic survival. This also means that the animal can run into the problem of self-pollution through the build-up of anaerobic end-products (Lutz, 1989). In contrast, depressing the metabolism clearly impairs the animal’s ability to respond to

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external stimuli. Deep metabolic depression leaves the animal defenceless against predators and unable to respond rapidly to changes in its environment.

The purpose of this review is to compare and contrast mechanisms that allow the brains of anoxia-tolerant vertebrates 'functionally' to survive anoxia.

Metabolic depression strategy

Degree of metabolic depression

Metabolic depression can be produced through behavioural as well as cellular adjustments in the rate of energy use. On the behavioural level, there is a striking difference in the response of *Carassius* and freshwater turtles to anoxia: the anoxic crucian carp reduces its spontaneous locomotor activity to about half that seen during normoxia (Fig. 1). By contrast, at room temperature, freshwater turtles appear completely limp within 30–60 min of the start of anoxia (the time it takes them to deplete their internal oxygen stores; Sick *et al.* 1982). That this inactive state is controlled by the brain is clearly demonstrated by the observation that the bodies of decapitated turtles (*Trachemys*) continue to move for more than 24 h although they are obviously completely deprived of oxygen (G. E. Nilsson and P. L. Lutz, unpublished observations).

Measurements of the heat production of anoxic goldfish and turtles also reveal differences. Anoxic goldfish depress their whole-body rate of heat production to about one-third of that seen during normoxia (van Waversveld *et al.* 1989), whereas anoxic turtles lower their heat production to one-tenth of the normoxic value (Jackson, 1968).

With regard to the brain, calculations based on changes in the levels of energy metabolites indicate that the anoxic turtle brain reduces its metabolic rate to around one-twentieth of the normoxic rate (Lutz *et al.* 1984; Chih *et al.* 1989*a*). Since anoxic *Carassius* remain responsive, it is highly unlikely that their brains are so deeply depressed. Microcalorimetric measurements of heat production by crucian carp brain slices show a 31 % reduction in ATP use in response to anoxia (Johansson *et al.* 1995). However, measurements on isolated brain slices are likely substantially to underestimate *in situ* metabolic depression because brain slices are already metabolically depressed (Lutz and Nilsson, 1994). Anoxic turtle brain slices only show a 40 % reduction in metabolic rate (Doll *et al.* 1994).

Mechanisms of metabolic depression

Channel arrest

Because the largest portion of the brain's energy budget is devoted to maintaining or restoring ion gradients, a reduction in ion flux through channel arrest could produce important energy savings (Hochachka, 1986). Sick *et al.* (1993) proposed more specifically that a net decrease in plasma membrane ion permeability during anoxia could be achieved *via* two distinct changes: (1) 'leakage arrest', whereby ion leakage is reduced when neurones are electrically inactive (i.e. not spiking), and

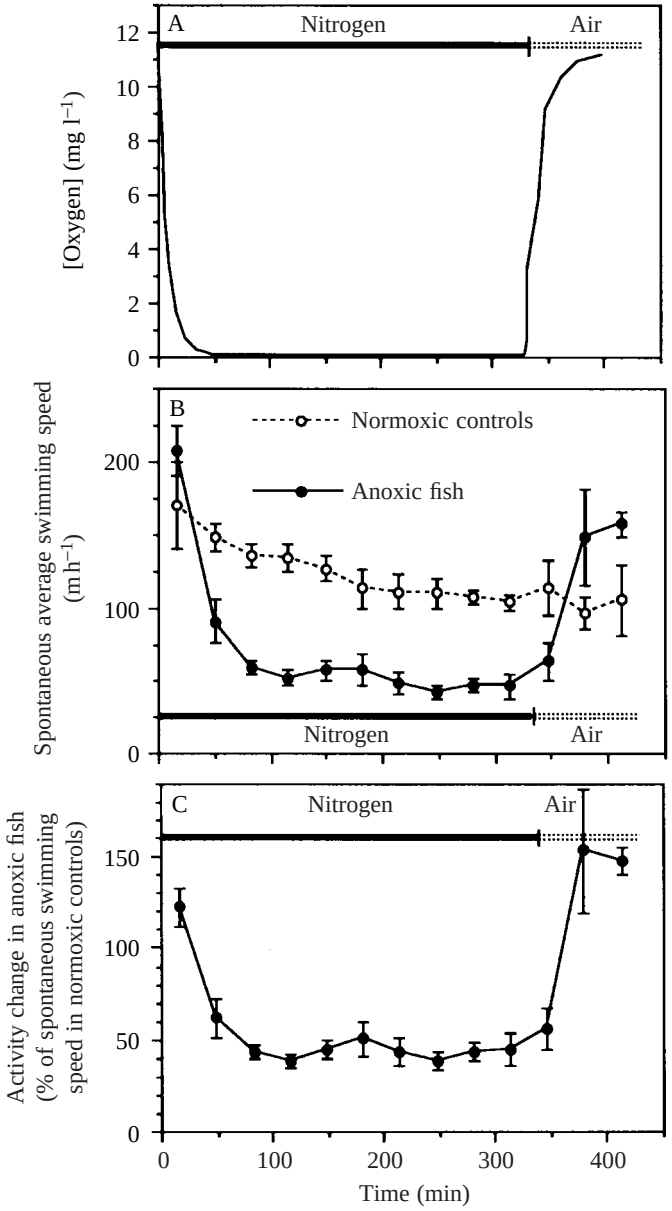

Fig. 1. Anoxic depression of spontaneous locomotor activity in crucian carp. Locomotor activity was measured using a computerized video system that tracked the spontaneous movement of the fish (from Nilsson *et al.* 1993). Values are means ± 95 % confidence intervals for five (anoxic) or six (normoxic) fish.

(2) 'spike arrest', which may be brought about by inhibiting channel activity associated with action potentials or by reducing their activity by suppressing synaptic transmission. There is evidence of a reduction in ion permeability in the anoxic turtle brain, mediated in part by adenosine; there is no evidence of such a down-regulation in the crucian carp.

K^+channels. Chih *et al.* (1989*b*) found that the rate of K^+ efflux from the anoxic turtle brain was substantially lower than that seen in the normoxic brain (Fig. 2C), the first indication of 'channel arrest' (Hochachka, 1986) in this system. There is recent evidence that this long-term decrease in K^+ leakage is partially mediated by adenosine receptors (Peck and Lutz,

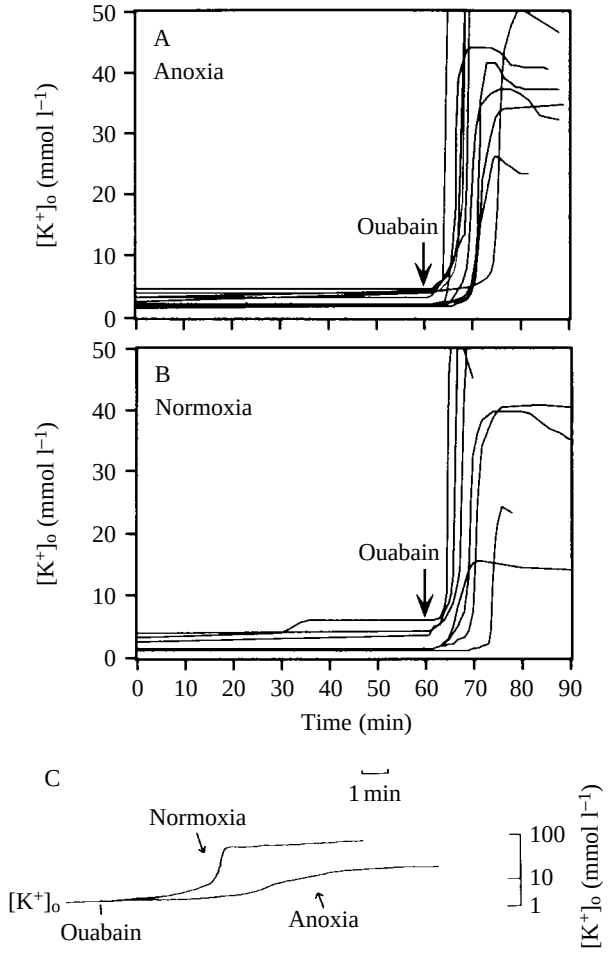

Fig. 2. Changes in extracellular $[K^+]$ in anoxic (A) and normoxic (B) crucian carp brain and turtle brain (C) during superfusion with ouabain (from Chih *et al.* 1989*b*; Johansson and Nilsson, 1995).

1996). In brains superfused with the specific adenosine A_1 blocker 8-cyclopentyltheophylline (8-CPT), the K^+ efflux during anoxia is significantly faster than that from untreated anoxic brains. By contrast, K^+ efflux rates were not diminished in the anoxic crucian carp brain, indicating that there had been no change in K^+ membrane permeability in this species (Fig. 2A,B) (Johansson and Nilsson, 1995).

In the mammalian brain, the initial response to anoxia is a rapidly induced short period of hyperpolarization, which has been attributed, depending on the brain region, to the *activation* of ATP-sensitive K^+ channels (K_{ATP} channels) (Krnjevic, 1993). Hyperpolarization would depress neuronal activity, by increasing the neuronal input needed to reach the action potential threshold, and thus provide an important, albeit temporary, protection. In this regard, the K_{ATP} channels are a sensitive early defence against incipient energy failure. By opening in response to a low ATP/ADP ratio and closing when this ratio is restored, they respond directly to changes in the intracellular energy charge.

However, K_{ATP} channels appear to be of little importance in the turtle and crucian carp brain. Using a specific blocker of mammalian K_{ATP} channels (glibenclamide), Jiang *et al.* (1992) could barely detect K_{ATP} channels in the turtle brain and found no signs of an opening of K_{ATP} channels during anoxia. Similarly, in anoxic as well as normoxic crucian carp, glibenclamide had no effect on the rate of neural K^+ efflux after the brain had been forced to depolarize by ouabain superfusion (Johansson and Nilsson, 1995).

The absence of K_{ATP} channels in anoxia-tolerant vertebrates is probably not simply a phylogenetic feature since glibenclamide-binding has been found in other ectothermic vertebrates (clawed frogs) (Jonas *et al.* 1991). Their absence in anoxia-tolerant vertebrates may be functional in that K^+-mediated hyperpolarization can only serve as a hypoxia defence mechanism in the short term (over minutes) and may be disastrous over longer anoxic periods. The increase in K^+ conductance will cause an increased efflux of K^+ and a gradual rise in extracellular $[K^+]$ with a concomitant depolarization. Indeed, this is what happens in the mammalian brain after a few minutes of anoxia.

Na^+ channels. In support of the suggestion that hypoxia-tolerant cells have inherent low channel densities (Hochachka, 1986), Edwards *et al.* (1989) found that the density of voltage-gated Na^+ channel in isolated nerve endings of turtle brain (synaptosomes) was one-third of that of rat synaptosomes. They concluded, however, that by itself this difference cannot be an important factor in the 100-fold difference in anoxia-tolerance. Xia and Haddad (1991) similarly found that for most brain regions, particularly the cerebellum, the Na^+ channel density of the turtle was much less than that of the rat, and reached the same conclusion.

In contrast, changes in Na^+ channel activity strongly affect metabolic rate. Closing the Na^+ channels by treatment with tetrodotoxin and increasing Na^+ channel activation by treatment with veratridine caused substantial decreases and increases, respectively, in synaptosome energy consumption (Edwards *et al.* 1989). The only direct evidence for the modulation of voltage-gated Na^+ channels in the turtle comes from a study which found that 4 h of anoxia produced a 42 % decline in the density of voltage-gated Na^+ channels in the isolated turtle cerebellum (Peréz-Pinzón *et al.* 1992*b*). Such a mechanism may underlie an anoxia-induced 14 mV increase in Na^+ action potential threshold in the isolated turtle cerebellum (Perez-Pinzon *et al.* 1992*a*). Interestingly, there is evidence that hypoxia reduces cell excitability in human neocortical brain slices by increasing the probability that Na^+ channels will be in the inactive state (Cummings *et al.* 1993).

Ca^{2+} channels. The uncontrolled inflow of Ca^{2+} into mammalian neurones through, for example the over-stimulated *N*-methyl-D-aspartate (NMDA) glutamate receptor, signals a wide variety of pathological processes, including the activation of proteases, lipases and nucleases, and is thought to be one of the principal causes of anoxic brain death (Szatkowski and Attwell, 1994). Thus, a down-regulation of Ca^{2+} channels could be of advantage during anoxia and, indeed, there is evidence that such a mechanism exists in turtles (Fig. 3). The anoxia-induced reduction in Ca^{2+} permeability appears to be adenosine-mediated (Buck and Bickler, 1995) and it may be

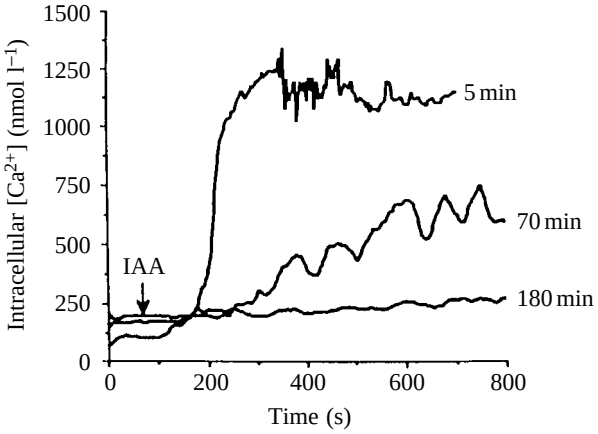

Fig. 3. Effects of iodoacetate (IAA) superfusion (to block anaerobic glycolysis) on intracellular Ca^{2+} concentration in turtle brain cortical slices. Slices were pre-exposed to 5, 70 or 180 min of anoxia. Anoxia results in a down-regulation of Ca^{2+} conductivity (from Bickler and Gallo, 1992).

induced by a soluble endogenous substance, possibly a protein (Bickler and Gallo, 1993).

To summarize ion channels: there is evidence that the anoxic turtle brain undergoes a reduction in ion membrane permeability (to K^+, Na^+ and Ca^{2+}), possibly through the involvement of adenosine, and that this decrease in ion permeability may not occur in the anoxic crucian carp brain. A reduction in ion permeabilities would produce energy savings, but results in a quiescent brain, a cost that the crucian carp might not be able to afford. However, the degree of channel arrest is probably slight. A substantial decrease in ion conductance would be expected to produce a corresponding increase in membrane input resistance. But Peréz-Pinzón *et al.* (1992*a*) found a slight decrease in input resistance in anoxic Purkinje cells in isolated turtle cerebellum, and Doll *et al.* (1991, 1993) could detect no change in whole-cell input resistance of turtle cortical pyramidal neurones during anoxia.

Adenosine

In the mammalian brain, extracellular adenosine level is greatly increased during anoxia or ischaemia (Newby *et al.* 1990), where it acts to produce an increase in cerebral blood flow (Collis, 1989) and to decrease neuronal excitability as well as to suppress excitatory neurotransmitter release (Prince and Stevens, 1992). Adenosine, in consequence, is thought to be an important endogenous protective agent during ischaemia and anoxia in mammals.

Adenosine receptors have been detected in fish brain (Siebenaller and Murray, 1986), and adenosine has been shown to inhibit neurotransmitter release in fish (Oshima, 1989). In the turtle, shortly after the onset of brain anoxia there is a substantial but temporary rise in extracellular adenosine level, which is probably linked to the simultaneous fall in ATP level (Nilsson and Lutz, 1992; Lutz and Nilsson, 1994). The hypothesis that adenosine plays a critical role in anoxia-tolerance is supported by the finding that superfusing the anoxic isolated turtle cerebellum with the adenosine receptor blockers theophylline or 8-CPT caused rapid depolarization (Peréz-Pinzón *et al.* 1993).

Although an increase in extracellular adenosine level has not been detected in the crucian carp brain, experimental evidence points to a role for adenosine in metabolic depression in these fish. Anoxic crucian carp given aminophylline to block their adenosine receptors excrete ethanol at a three times the rate of normoxic individuals, while the same dose of aminophylline ($75\,mg\,kg^{-1}$) does not increase the rate of O_2 consumption during normoxia (Nilsson, 1991). In these experiments, aminophylline was administered systemically (intraperitoneally), so the effects may involve central as well as peripheral adenosine receptors.

Neurotransmitters

In the mammalian brain, anoxia produces a massive release of the excitatory neurotransmitter glutamate, with toxic effects. By contrast, there is little or no release of glutamate in the brain of anoxic turtles (Nilsson and Lutz, 1991) or crucian carp (P. Hylland and G. E. Nilsson, unpublished results).

There is an increase in the extracellular level of γ-aminobutyric acid (GABA) in the anoxic turtle brain and anoxic crucian carp brain (Nilsson and Lutz, 1991; P. Hylland and G. E. Nilsson, unpublished results). GABA has similar inhibitory actions in turtle and mammalian brain (Kriegstein and Connors, 1986), and a role for GABA in the depression of the systemic metabolic rate of anoxic crucian carp has been described (Nilsson, 1991).

Turtle and rat brains have similar densities of $GABA_A$ receptors (Lutz and Leone-Kabler, 1995), and these $GABA_A$ binding sites show identical regulatory and kinetic properties (Sakurai, 1991). There is an increase in $GABA_A$ receptor number within 2 h of anoxia, and numbers continue to increase for at least 24 h (Sakurai *et al.* 1993; Lutz and Leone-Kabler, 1995). This up-regulation of $GABA_A$ receptors may function to increase the effectiveness of the inhibitory action of the GABA that is released during anoxia. Such an effect could be further enhanced by the decreasing extracellular pH that occurs during anoxia, since protons have a facilitatory action on $GABA_A$-receptor-mediated responses (Kaila, 1994). An up-regulation of postsynaptically located $GABA_A$ receptors has also been detected in the rat brain during hypoxia (Ninomiya *et al.* 1988).

Electrical depression

Perhaps the most significant result of the above mechanisms is a reduction in brain electrical activity. Since electrical activity is responsible for about 50 % of the energy consumption of the mammalian brain, making it electrically quiescent can produce considerable energy savings (Lutz and Nilsson, 1994). Indeed, electrical activity may account for an even greater proportion of the energy consumption of the ectotherm brain, since the brain spends less energy in counteracting the ion-leak fluxes that underlie heat production in endotherms (Else and Hulbert, 1987).

In the turtle brain, there is a drastic fall in electrical activity during the transition to the full anoxic state (Sick *et al.* 1982) and, after about 100 min of anoxia, the root mean square (RMS) voltage of the electroencephalogram levels out at a depressed floor activity level of about one-fifth of normoxic levels, where it remains throughout the rest of the anoxic episode (G. E. Fernandez and P. L. Lutz, unpublished data). Evoked potentials are also depressed to about 20–50 % of normoxic levels during anoxia, indicating a corresponding reduction in synaptic transmission (Feng *et al.* 1988; Perézz-Pinzón *et al.* 1992*b*).

This degree of electrical depression results in a comatose-like state in the anoxic turtle and may represent the minimal electrical activity required to ensure the survival of the brain as an integrated unit; it is not likely to be compatible with the active behaviour of the anoxic crucian carp.

The glycolytic strategy

Apart from metabolic depression, the other option available to an animal to survive anoxia is to increase its glycolytic flux substantially. As the crucian carp and the goldfish are much more metabolically active during anoxia than is the turtle, the genus *Carassius* may have a greater reliance on glycolytic ATP production which, indeed, appears to be the case.

Glycogen stores

The crucian carp has the largest store of glycogen of any vertebrate. At the beginning of winter, 15 % of its body mass is liver and 30 % of the liver mass is glycogen (Hyvärinen *et al.* 1985). The amount of glycogen stored by the turtle, while still impressive, is somewhat less, accounting for 15 % of the mass of the liver of the freshwater turtle (Clark and Miller, 1973).

Brain glycogen concentrations of crucian carp, goldfish and freshwater turtle are also higher than those of other vertebrates, ranging between 12.8 and 19.5 μmol hexose g^{-1}, while the brain glycogen content of anoxia-sensitive species such as the rat, mouse and rainbow trout is only 2.2–3.7 μmol g^{-1} (for a review, see Lutz and Nilsson, 1994).

In the crucian carp, the brain glycogen level falls by 75 and 95 % during the first 2 and 7 h of anoxia, respectively (Schmidt and Wegener, 1988). Thus, brain glycogen may be responsible for brain glucose supply during the initial phase of anoxia, but the huge liver glycogen store has to take over this responsibility when the brain glycogen reserves have been depleted.

Increased blood glucose level and brain blood flow

In order for the anoxic brain to use the glucose stored in the liver, the delivery rate of glucose to the brain must increase. This can be accomplished by increasing the concentration of glucose in the blood or by increasing the rate of blood supply to the brain. Both mechanisms are utilized by anoxia-tolerant vertebrates.

In goldfish, the blood glucose concentration increases fivefold during anoxia (Shoubridge and Hochachka, 1980); in freshwater turtles (*Chrysemys* and *Trachemys*) held at room temperature, anoxia also produces a marked hyperglycaemia, with plasma glucose concentration rising from 3 mmol l^{-1} to as high as 25 mmol l^{-1} (Penney, 1974).

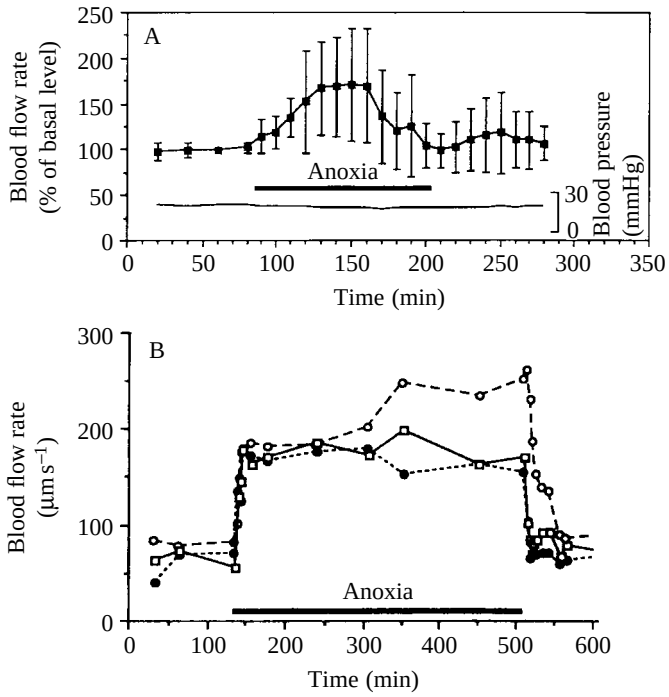

Fig. 4. The effect of anoxia on brain blood flow in the turtle (A) and crucian carp (B). Note that, in the turtle, the increased blood flow rate during anoxia is only temporary (from Hylland *et al.* 1994; Nilsson *et al.* 1994). 1 mmHg=0.133 kPa. In A, values are means ± S.E.M., *N*=5. In B, results for three carp are given.

In crucian carp, the brain blood flow rate more than doubles during anoxia and remains elevated for the entire anoxic period (Fig. 4B) (Nilsson *et al.* 1994). A similar increase in blood flow also occurs in the anoxic freshwater turtle brain (Fig. 4A) (Hylland *et al.* 1994), but here the increase is only temporary, and the blood flow starts to fall back to pre-anoxic values within 100 min. Thus, in the turtle the brain, blood flow increase may be an emergency response that is turned off as soon as metabolic depression sets in and energy balance is restored. The cerebral blood flow responses of both crucian carp and turtles appear to be mediated by adenosine, since the anoxia-induced increase in blood flow can be inhibited by aminophylline in both species and a similar elevation of brain blood flow can be provoked by superfusing the brain of crucian carp or freshwater turtles with adenosine (Nilsson *et al.* 1994; Hylland *et al.* 1994).

Ethanol production – the new opportunity

Lactate is the end-product of glycolysis for all vertebrates, with one exception: the genus *Carassius*. These fish produce ethanol as the major glycolytic end-product during anoxia. By releasing the ethanol to the water through the gills, the crucian carp avoids the problem of self-intoxication and end-product

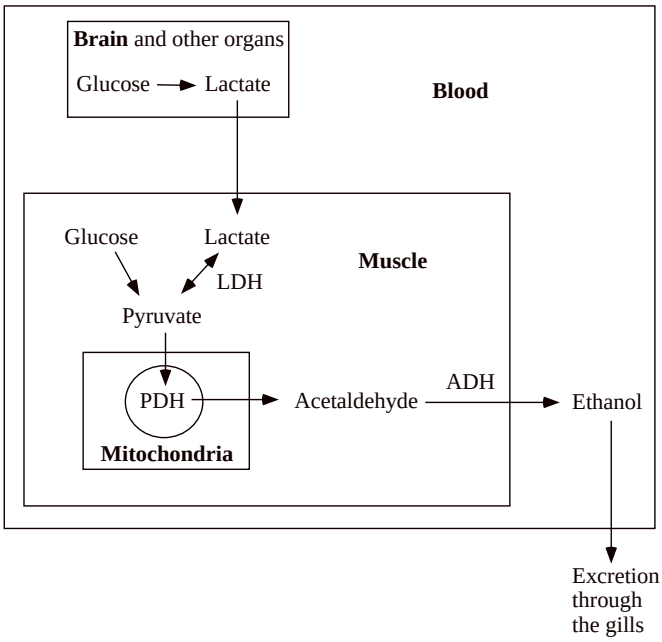

Fig. 5. Ethanol-producing pathway in the genus *Carassius*. By releasing ethanol into the water, crucian carp and goldfish avoid a build-up of anaerobic end-product. LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase; PDH, pyruvate dehydrogenase.

build-up, and the high lactate levels and consequent acidosis faced by other vertebrates. The ability to produce ethanol in combination with the huge glycogen stores allow *Carassius* to maintain a high glycolytic rate for long periods without having to suffer from enormous lactate loads – an opportunity that is not available to the turtle.

The ethanol-producing pathway of *Carassius* is summarized in Fig. 5. The unique part of the pathway starts inside the mitochondria, where pyruvate is thought to be decarboxylated to form acetaldehyde and CO_2 in a reaction catalyzed by pyruvate dehydrogenase (van Waarde *et al.* 1993). This enzyme occurs in all vertebrates as part of the pyruvate dehydrogenase complex, which converts pyruvate to acetyl-coenzyme A for delivery to the citric acid cycle. In all other vertebrates, however, acetaldehyde is only an intermediate in the reactions catalyzed by this enzyme complex, and acetaldehyde is never allowed to leave the complex. Thus, in *Carassius*, the pyruvate dehydrogenase complex appears to 'leak' during anoxia, allowing the intermediate acetaldehyde to slip out. Acetaldehyde then diffuses out of the mitochondria into the cytosolic compartment, where it is rapidly converted to ethanol by alcohol dehydrogenase (ADH) – an enzyme present in all vertebrates, although it occurs at elevated levels in *Carassius* (Shoubridge and Hochachka, 1980). Moreover, ADH has a quite unique distribution in the tissues of *Carassius*. In other vertebrates, the highest activities of ADH are always found in the liver, whereas in *Carassius*, ADH predominates in skeletal muscle (Nilsson, 1988).

It is quite possible that the modification to the pyruvate dehydrogenase complex is the result of a single mutation – a mutation that suddenly opened up a new avenue for anoxic survival and freed the animals from the necessity of entering into deep metabolic depression during prolonged anoxia. The unique tissue distribution of ADH in *Carassius* may be an additional modification that occurred later. Fish skeletal muscle, which is by far the largest body organ, may be a more efficient site than the liver for turning lactate rapidly into ethanol. A more detailed discussion of the distribution of ADH in vertebrates and its consequence for anoxic ethanol production has been given by Nilsson (1988).

However, other tissues in *Carassius*, including the brain, lack ADH (Shoubridge and Hochachka, 1980; Nilsson, 1988), so that lactate must still be the major anaerobic end-product in brain. In fact, brain slices from crucian carp release lactate to the bathing solution during anoxia and are unable to produce ethanol (Johanssen *et al.* 1995). The lactate produced by the brain is transported by the blood to the muscles, where it is transformed to ethanol and CO_2.

By contrast, freshwater turtles have to cope with enormous lactate levels during prolonged anoxia. In the turtle *Chrysemys picta*, for example, plasma lactate levels can rise to as high as 200 mmol l^{-1} during 3 months submerged in cold (3 °C) anoxic water (Ultsch and Jackson, 1982). The increased lactate is accommodated by substantial increases in blood Mg^{2+} and Ca^{2+} concentrations (up to 25 times normoxic control levels) mobilized from skeletal sources (Warburton and Jackson, 1995).

Carassius versus turtle: glycolytic activation *versus* glycolytic depression

As mentioned above, turtle brain blood flow is only temporarily increased during anoxia, indicating that the activation of glycolysis is just an initial emergency response. This scenario fits well with biochemical studies suggesting that, after an hour or so of anoxia, key enzymes associated with glycolysis are in less active forms (Kelly and Storey, 1988). Phosphofructokinase, in particular, shows increased inhibition by ATP and citrate, and a decreased sensitivity to its activator AMP during this period, and glycogen phosphorylase-a activity is reduced by 70 %, indicating a decrease in the breakdown of glycogen to glucose (Kelly and Storey, 1988). The covalent modification of key glycolytic enzymes by phosphorylation may play a role in coordinating the depression of glycolysis in the turtle brain, by producing less active forms of the enzymes (Storey, 1996). The fall in pH that accompanies prolonged anoxia can also act to depress enzyme activities, particularly of those associated with glycolysis (Storey, 1996).

In contrast to the turtle, crucian carp brain slices show a sustained 2.4-fold increase in the rate of glycolysis during anoxia (Johansson *et al.* 1995), and even after 24 h of anoxia (at 7 °C), there is a 3.5-fold increase in the level of fructose 2,6-bisphosphate, a phosphofructokinase activator (Storey, 1987). This is combined with an increase in the level of the active (phosphorylated) (a) form of glycogen phosphorylase in brain, while the active form of this enzyme was suppressed in

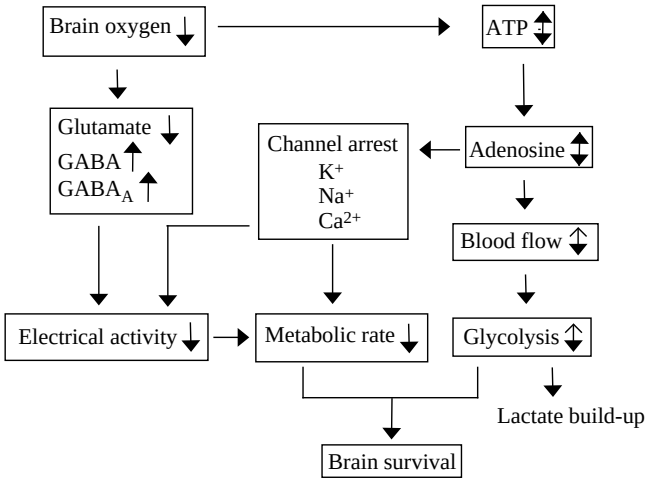

Fig. 6. Model of the events leading to anoxic brain survival in the turtle. Energy insufficiency in the anoxic turtle brain provokes a release of adenosine, which facilitates an increase in brain blood flow and enhances anaerobic glycolysis. But this lactate-producing strategy is limiting, and an early down-regulation of the brain metabolic rate follows, paralleled by a reduction in brain blood flow and a decreased activation of key glycolytic enzymes. Brain metabolic depression is brought about by invoking channel arrest, in part mediated by adenosine, the release of inhibitory GABA and the up-regulation of $GABA_A$ receptors. The result is a suppression of neurotransmission and a substantial depression in electrical activity to minimal levels.

all other tissues examined (Storey, 1987). There is also evidence that protein synthesis is not reduced in the anoxic crucian carp brain (R. Smith, D. Houlihan and G. E. Nilsson, unpublished results) but may be in the anoxic turtle brain (ongoing studies).

It is the exhaustion of the liver glycogen store that appears to be the limiting factor for anoxic survival in *Carassius*. It is likely, therefore, that when faced with anoxia *Carassius* rapidly down-regulates its metabolic rate to a degree that is still compatible with maintaining activity and responsiveness to external stimuli but, at the same time, substantially lowers its rate of depletion of glycogen stores.

Summary and conclusions

The contrasting strategies of the turtle and *Carassius* represent two divergent adaptations for anoxic brain survival: in one, there is a maximal shut down to deep metabolic depression (Fig. 6): in the other, glycolysis is enhanced to allow coordinated activity during anoxia The first produces a comatose brain, the second one that is still 'functional'. The mechanisms that result in a suppression of neurotransmission and electrical activity in the anoxic turtle brain are illustrated in Fig. 6. The key adaptation that allows a continued high level of glycolysis in the carp is ethanol production, which avoids the self-pollution of lactate produced during glycolysis.

The selection pressure on turtles to suppress their metabolism must be very strong since they have to cope with enormous lactate loads, and any increase in metabolic rate during anoxia would shorten the anoxia-endurance of the overwintering turtle. The switch to ethanol production, therefore, liberated the crucian carp from the path taken by anaerobic lactate producers to an increasingly suppressed metabolism. This may have arisen from a single mutation that made the pyruvate dehydrogenase complex start to leak acetaldehyde. However, one significant drawback of the ethanol strategy is that ethanol excretion represents a loss of energy substrate. For *Carassius*, the benefits gained from remaining active during anoxia could be well worth this energy loss. Levels of dissolved oxygen can vary widely in a freshwater habitat and, for a water-breather, being active during anoxia allows you to go to the oxygen rather than to have to wait for the oxygen to come to you.

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