

EFFECTS OF ANOXIA ON ENERGY METABOLISM IN CRUCIAN CARP BRAIN SLICES STUDIED WITH MICROCALORIMETRY

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Summary

Crucian carp (*Carassius carassius* L.) is an exceptionally anoxia-tolerant vertebrate. To determine whether isolated crucian carp brain tissue survives anoxia and whether it displays anoxic metabolic depression, heat production (using microcalorimetry), lactate production, ethanol production and the maintenance of ATP, ADP and AMP levels and energy charge were measured in telencephalic brain slices during anoxia. In response to anoxia, heat output decreased by 37%, corresponding to a 31% fall in ATP turnover rate. Adenylate phosphates and energy

charge were well maintained and no ethanol was produced during anoxia. It is concluded that crucian carp brain tissue has an intrinsic capacity to tolerate anoxia and that it responds to anoxia by depressing metabolic rate and elevating the glycolytic rate, thereby maintaining ATP levels.

Key words: anoxia, brain slices, calorimetry, *Carassius carassius*, crucian carp, energy metabolism, ethanol production, lactate production, metabolic depression.

Introduction

The crucian carp (*Carassius carassius*) is one of the very few vertebrates that can survive days of anoxia at room temperature and several months at temperatures close to 0°C (Piironen and Holopainen, 1986). The closely related goldfish (*C. auratus* L.) is also highly anoxia-tolerant, and a similar degree of anoxia tolerance has evolved, obviously separately, in some freshwater turtles of the genera *Chrysemys* and *Trachemys* (Ultsch, 1989).

During anoxia, vertebrate cells can only produce ATP by anaerobic glycolysis, a process that yields 2 mol ATP per mol glucose. This should be compared with a yield of approximately 25 mol ATP per mol glucose during aerobic glucose oxidation (Hochachka and Somero, 1984). Thus, producing ATP at sufficient rates is a fundamental problem for an anoxic animal. Because of its high energy requirements, the brain is normally very sensitive to anoxia. Thus, much research has focused on mechanisms for brain survival in anoxia-tolerant vertebrates (Lutz and Nilsson, 1994).

Anoxia-tolerant vertebrates appear to utilise two different strategies simultaneously to provide protection against a loss of ATP: (1) increasing the glycolytic flux (Pasteur effect) and (2) reducing the rate of ATP consumption (metabolic depression). The glycolytic adaptations to anoxia that have been found in the genus *Carassius* include the ability of skeletal muscles to produce ethanol as the main metabolic end product (Shoubridge and Hochachka, 1980), thereby avoiding lactic acidosis. Moreover, glycolytic ATP production in *Carassius* is supplied with glucose from the largest liver

glycogen store found in any vertebrate (Hyvärinen *et al.* 1985). The anoxic brain receives additional glucose from this store through a doubling of brain blood flow rate (Nilsson *et al.* 1994). However, a total depletion of the liver glycogen store is what finally limits the anoxic survival of crucian carp (Nilsson, 1990). Thus, anoxic survival time will strongly correlate with the ability to depress the metabolic rate.

For turtles, recent studies on isolated brain tissue (brain slices) have shown that their brain has an intrinsic capacity to tolerate anoxia (Pérez-Pinzón *et al.* 1992b), and microcalorimetric measurements on turtle brain slices have demonstrated metabolic depression during anoxia (Pérez-Pinzón *et al.* 1991; Doll *et al.* 1994). However, it has hitherto not been shown that crucian carp or goldfish brain tissue is anoxia-tolerant *per se* or displays metabolic depression. Since these fishes are the only other notable examples of anoxia-tolerant vertebrates, information is urgently needed on the anoxic responses of an isolated brain preparation of *Carassius*. Moreover, since *Carassius* shows a systemic metabolic depression of 70% (Van Waverveld *et al.* 1989), it is of interest to determine whether metabolic depression in the whole animal is paralleled by shifts in heat production in isolated tissues.

The aim of the present study was to determine whether telencephalic slices from crucian carp have the ability to tolerate anoxia and whether anoxic metabolic depression is displayed by this tissue. This was accomplished by measuring the effect of anoxia on heat production (using

microcalorimetry), lactate production, ethanol production and the maintenance of ATP, ADP and AMP levels and energy charge.

Materials and methods

Animals

Crucian carp (*Carassius carassius* L.), weighing 10–30 g, were caught in a pond near Uppsala in August. They were kept indoors in 500 l tanks (100 fish in each) continuously supplied (21 min^{-1}) with aerated water (12°C), and they were fed daily with commercial trout food (Ewos, Sweden). The artificial light automatically followed the light/dark cycle of Hamburg's latitude and longitude.

Brain slice preparation

The fish was decapitated and the skull was opened dorsally. The telencephalic hemispheres were gently removed after the olfactory tracts and the caudal connections to the diencephalon had been cut with a pair of fine scissors. This procedure required less than 1 min. The telencephalic hemispheres were glued to a Teflon specimen holder with cyanoacrylate adhesive and sliced using a Vibratome (Vibroslice 752 from World Precision Instruments, Sarasota, FL) to a thickness of $300 \mu\text{m}$ while submerged in ice-cold artificial cerebrospinal fluid (aCSF).

The aCSF, adopted from Richards (1981), consisted of 137 mmol l^{-1} NaCl, 2 mmol l^{-1} KCl, 1.25 mmol l^{-1} KH_2PO_4 , 1.1 mmol l^{-1} MgSO_4 , 1.1 mmol l^{-1} CaCl_2 , 16 mmol l^{-1} NaHCO_3 and 20 mmol l^{-1} glucose. The aCSF was bubbled with O_2 or N_2 for at least 60 min, and an O_2 electrode was then used to check that the O_2 content was 100% or below 1% of saturation, respectively. The pH of the aCSF was adjusted to 7.4 with HCl just before the experiment. A glucose concentration of 20 mmol l^{-1} was used because blood glucose can reach this level in anoxic crucian carp (D. Johansson and G. E. Nilsson, unpublished observations) and because Pérez-Pinzón *et al.* (1992b) found 20 mmol l^{-1} glucose in the aCSF to be necessary for anoxic survival of cerebellar slices from anoxia-tolerant freshwater turtles.

Pairs of slices were put in 3 ml crimp-cap glass-ampoules with a rubber septum (Thermometric, Järfälla, Sweden) filled with ice-cold oxygenated or anoxic aCSF under an atmosphere of O_2 or N_2 . After the ampoules had been sealed, a syringe needle was used to introduce a $100 \mu\text{l}$ bubble of O_2 or N_2 at the top of the ampoules. These ampoules were used both for microcalorimetry and for the incubations preceding metabolite measurements.

Microcalorimetry

Heat production measurements were carried out using a heat conduction multichannel microcalorimeter [Thermal Activity Monitor 2277, from Thermometric (formerly LKB), Järfälla, Sweden]. Static calibration was used, and baseline fluctuations were less than $0.25 \mu\text{W}$ over 24 h in the $10 \mu\text{W}$ range. All

measurements were performed at 12°C . The heat production of the slices was either measured for 20 h in oxygenated Ringer or for 20 h in anoxic Ringer followed by 20 h in oxygenated aCSF. The reference ampoules also contained anoxic or oxygenated aCSF. When anoxic aCSF was replaced by oxygenated aCSF, both the measurement and reference ampoules were lifted out of the measurement chamber and the N_2 -bubbled aCSF was carefully removed and fresh oxygenated aCSF was added.

Before all measurements, the ampoules were kept in the heat-equilibrating position of the calorimeter for 60 min (to allow them to warm up from 0°C to 12°C) before they were lowered into the measuring chambers. After the ampoules had been placed in the measuring chambers, control experiments (both ampoules containing aCSF but no slices) showed that it took 70–110 min for the system to equilibrate and to show a steady zero line (the variation in time probably largely depending upon how much friction heat the ampoules had accumulated when they were inserted in the measuring chambers). Therefore, heat output measurements from the first 120 min were disregarded.

The brain slices were weighed after the microcalorimetry measurements. There were no significant difference in masses between anoxic ($1.9 \pm 0.3 \text{ mg}$) and oxygenated ($1.7 \pm 0.2 \text{ mg}$) brain slices.

Metabolite measurements

Pairs of slices were incubated in 3 ml vials (see above) in a waterbath at 12°C in four different ways: (1) 1 h in oxygenated aCSF, (2) 20 h in oxygenated aCSF, (3) 20 h in anoxic aCSF and (4) 20 h in anoxic aCSF followed by replacement of the aCSF with oxygenated aCSF and an additional 10 h of incubation. After the incubations, the slices were immediately frozen in liquid N_2 . The aCSF in the vials was saved for lactate and ethanol analysis. In addition, the telencephalons from six fish were removed and immediately frozen (within 10 s of decapitation) in liquid N_2 in order to obtain *in situ* values of adenosine phosphate concentrations.

While still frozen, the slices (or telencephalon) were weighed and sonicated ($3 \times 15 \text{ s}$) in $300 \mu\text{l}$ of 4% (w/v) ice-cold perchloric acid (PCA) using an MSE 100W ultrasonic disintegrator. These PCA homogenates were centrifuged at $15\,000 \text{ g}$ for 5 min. The resultant supernatants were neutralised with $200 \mu\text{l}$ of 0.85 mol l^{-1} K_2CO_3 and then centrifuged again at $15\,000 \text{ g}$ for 5 min. The levels of ATP, ADP and AMP in these supernatants were analysed using HPLC as described by Van der Boon *et al.* (1992). Lactate was analysed with Sigma Lactate Reagent kit 735-10, and ethanol was analysed enzymatically as described by Krebs *et al.* (1969). Energy charge was calculated according to the following formula:

$$\text{Energy charge} = ([\text{ATP}] + 1/2[\text{ADP}]) / ([\text{ATP}] + [\text{ADP}] + [\text{AMP}]).$$

Statistics

Data were tested with Kruskal–Wallis analysis of variance

followed by Wilcoxon rank test (two-tailed). All values presented are means \pm S.E.M. from six animals (six pairs of slices).

Results

The heat production of oxygenated and anoxic crucian carp brain slices is shown in Fig. 1. After 20h, the aCSF in the anoxia group was exchanged for oxygenated aCSF, and the heat production was measured for an additional 20h period. After an initial period of relatively rapidly falling heat production rates, the slices showed a slow steady decline in heat production of about $1\% \text{ h}^{-1}$. The slope of this decline did not differ significantly between anoxic ($1.02 \pm 0.22\% \text{ h}^{-1}$) and oxygenated ($1.11 \pm 0.31\% \text{ h}^{-1}$) slices and it may reflect a slow loss of viable cells. After 10h, the heat production of oxygenated slices was $758 \pm 62 \mu\text{W g}^{-1}$, while that of anoxic slices was $478 \pm 25 \mu\text{W g}^{-1}$ ($P=0.0022$), suggesting that anoxia caused a $37 \pm 9\%$ decrease in heat production.

A similar estimate for depression of heat production ($36 \pm 8\%$) was obtained when the total amounts of heat produced during 20h were calculated (corresponding to the areas under the curves in Fig. 1). These values were $55.5 \pm 4.1 \text{ J g}^{-1}$ for the oxygenated slices and $35.4 \pm 1.8 \text{ J g}^{-1}$ for the anoxic slices.

When the anoxic aCSF was exchanged for oxygenated aCSF, the heat production returned to the level that would be expected if the slow steady decline in heat production were taken into account (Fig. 1). When extrapolating back to the 10h values, an aerobic heat production value of $799 \pm 35 \mu\text{W g}^{-1}$ was obtained. This value was not significantly different from that of the brain slices kept in oxygenated aCSF for 10h ($758 \mu\text{W g}^{-1}$, see above) and suggested that the degree of metabolic depression was $40 \pm 3\%$ (the lowest and probably best estimate of the variance in metabolic depression, since each ampoule with slices was its own control). However, initially, the heat production of all the reoxygenated slices showed an overshoot over baseline values that lasted longer ($8.8 \pm 0.7\text{h}$ before returning to baseline) than the initial elevation seen in the oxygenated controls ($5.6 \pm 0.9\text{h}$, $P=0.015$) and, although not significant, it also seemed to be higher ($35.8 \pm 9.0\%$ over baseline after 2h) than in the oxygenated controls ($17.8 \pm 3.9\%$). We cannot calculate the total heat (area under curve) of the overshoots since most probably occurred during equilibration of the calorimeter.

Fig. 2 shows the total amount of lactate released to the aCSF by aerobic and anaerobic brain slices. These lactate values are given in relation to the slice mass and corresponded to lactate concentrations in the aCSF of $0.2\text{--}0.4 \text{ mmol l}^{-1}$ (oxygenated aCSF) and $0.4\text{--}0.9 \text{ mmol l}^{-1}$ (anoxic aCSF). Fig. 2 also shows that no ethanol could be found in the aCSF after 20h in anoxic or oxygenated aCSF. The detection limit of the ethanol assay was $10 \mu\text{mol l}^{-1}$.

From the value of total heat produced during 20h in anoxia (35.4 J g^{-1}) and the amount of lactate produced during the same period ($524 \mu\text{mol g}^{-1}$), a value of 68 kJ mol^{-1} of lactate

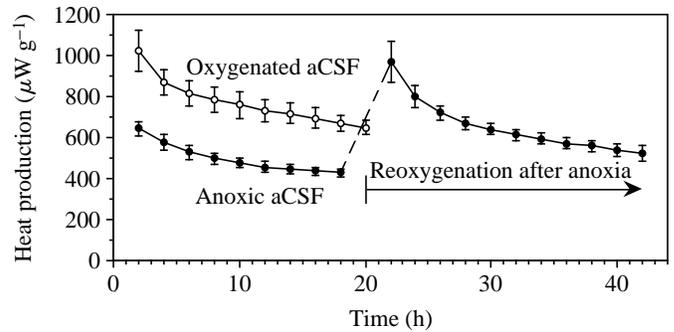


Fig. 1. Heat production by oxygenated and anoxic telencephalic brain slices from crucian carp. The anoxic artificial cerebrospinal fluid (aCSF) was exchanged for an oxygenated aCSF after 20h, and the heat production was measured for an additional 20h. Values are means \pm S.E.M. from six animals.

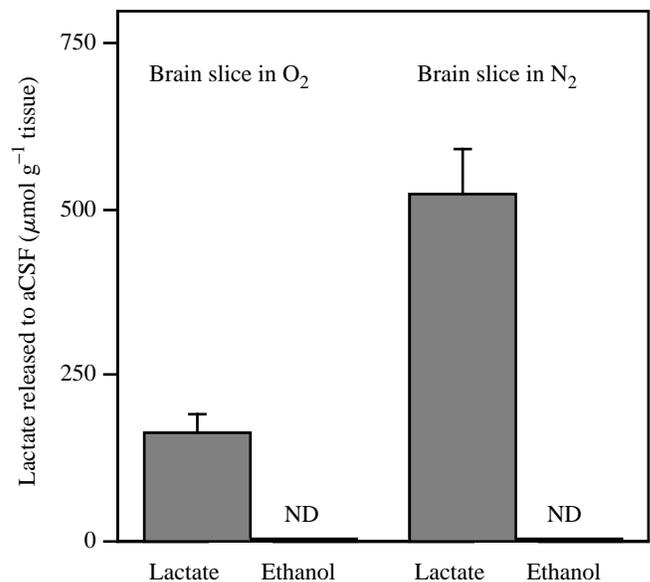


Fig. 2. Amount of lactate and ethanol produced by telencephalic brain slices and found in the aCSF after 20h in oxygenated aCSF or 20h in anoxic aCSF. ND, below detection limit. Values are means \pm S.E.M. from six animals

can be estimated for the catabolism of glucose to lactate. Considering that the volume of the slices was only about $1/1000$ of the aCSF volume, we have in this calculation assumed that the amount of lactate present in the slices was negligible. Indeed, no lactate could be detected in the slices. The detection limit for lactate in this small amount of tissue was approximately 10 mmol l^{-1} . It should be noted that the lactate produced must arise almost entirely from exogenous glucose, since the crucian carp brain only contains glycogen corresponding to about $20 \mu\text{mol glucose g}^{-1}$ (Schmidt and Wegener, 1988).

AMP, ADP, ATP, total adenylate phosphates (AMP+ADP+ATP) and energy charge were not significantly different ($P>0.2$) between slices subjected to 1h in oxygenated

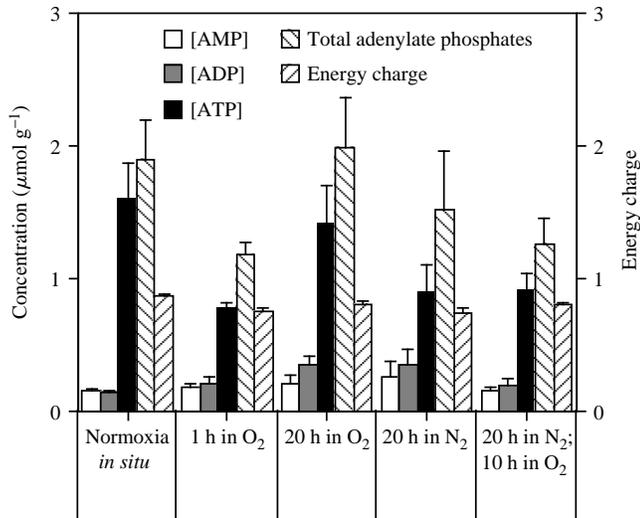


Fig. 3. Concentrations of AMP, ADP, ATP, total adenylate phosphates (AMP+ADP+ATP), and energy charge *in situ* and in brain slices kept in oxygenated aCSF for 1 h or 20 h, in anoxic (N₂-bubbled) aCSF for 20 h and in anoxic aCSF for 20 h followed by oxygenated aCSF for 10 h. Values are means + S.E.M. from six animals.

aCSF, 20 h in oxygenated aCSF, 20 h in anoxic aCSF or 20 h in anoxic aCSF followed by 10 h in oxygenated aCSF (Fig. 3). However, the energy charge was higher *in situ* than in the slices kept in oxygenated aCSF for 1 h ($P=0.009$), and a similar tendency was seen in the levels of ATP and total adenylate phosphates when comparing these two groups ($P=0.13$ for both parameters). However, these differences seemed to disappear when the slices had been incubated for 20 h.

Discussion

Maintenance of ATP levels and energy charge

The slices were in good condition energetically, since after 20 h, their energy charge and adenylate levels were not significantly different from the *in situ* values. Most importantly, ATP levels and energy charge were equally well maintained in both anoxic and oxygenated aCSF. Turtle brain slices (Doll *et al.* 1991; Pérez-Pinzón *et al.* 1992b) are the only brain slice preparations showing a similar anoxia tolerance, while rat brain slices lose most of their ATP within 5–10 min of anoxia (Lipton and Whittingham, 1984; Kass and Lipton, 1989). This clearly demonstrates that the anoxic survival of the crucian carp involves an intrinsic capacity of the brain to cope with anoxia.

Metabolic depression

The 37% decrease in heat production during anoxia shows that the crucian carp brain slices become metabolically depressed. Because anaerobic ATP production (from glucose to lactate) yields approximately 9% less heat than ATP production from aerobic glucose oxidation (Gnaiger, 1983), a 37% decrease in heat production during anoxia corresponds to a 31% fall in ATP turnover. These are low values

compared with the 70% decrease in heat production seen in whole bodies of the closely related goldfish (Van Waversveld *et al.* 1989). Interestingly, similar to the present results, the two studies on anoxia-tolerant turtle brain slices (Pérez-Pinzón *et al.* 1991; Doll *et al.* 1994) have both indicated a 30–40% fall in heat production during anoxia, although brain lactate accumulation *in situ* suggests a much deeper metabolic depression (Chih *et al.* 1989), and anoxic turtles display a 90% decrease in heat production at the whole-body level (Jackson, 1968). It is possible that the brain, or at least the telencephalon, is less metabolically depressed than the whole body during anoxia. However, a more likely explanation is that the metabolism of brain slices is partially depressed from the beginning. In mammals, the O₂ consumption rate of brain slices is only approximately 50% of that of the intact brain (Lipton and Whittingham, 1984), possibly because spontaneous electrical activity is absent (Astrup *et al.* 1981; Erecinska and Silver, 1989). Several lines of evidence suggest that anoxia-tolerant vertebrates such as the crucian carp and freshwater turtles down-regulate electrical activity in the brain during anoxia and that this is the major neural mechanism for metabolic depression (see Lutz and Nilsson, 1994, for a review). In anoxia-tolerant vertebrates, anoxia causes increased brain levels of GABA and adenosine, two inhibitory neurotransmitters/neuromodulators, which is likely to mediate a suppression of electrical activity (Nilsson, 1990; Nilsson and Lutz, 1992, 1993), and pharmacological blockades of GABA or adenosine receptors appear to inhibit metabolic depression in crucian carp (Nilsson, 1991, 1992). Interestingly, since any portion of metabolic depression based on lowered electrical activity would probably not be revealed by slice experiments, the present results suggest that the crucian carp brain has other mechanisms for making considerable energy savings than depressing electric activity. This could include suppressed protein synthesis. A decrease in ion-leak fluxes seems less likely, since membrane resistance does not increase in turtle brain slices (Doll *et al.* 1991; Pérez-Pinzón *et al.* 1992a).

It should also be pointed out that we avoided introducing gas mixtures in the experiments, only exposing the slices to pure O₂ or N₂. Brain slices from mammals as well as turtles are usually kept in a mixture of 95% O₂ and 5% CO₂ (Richards, 1981; Pérez-Pinzón *et al.* 1992b). However, a CO₂ level of 5% is much higher than what could be expected in fish tissues. Moreover, since an elevated P_{CO_2} has been suggested to mediate metabolic depression in some animals (Lutz, 1989), we decided to avoid any exogenous addition of CO₂. Still, it is unlikely that increased P_{CO_2} *per se* would be an important mediator of anoxic metabolic depression in *Carassius*, since this genus shows no increase, or only a slight increase, in CO₂ production in anoxia compared with normoxia (Van Waversveld *et al.* 1989) and does not become acidotic in anoxia (Van Waarde *et al.* 1993). Nevertheless, possible effects of endogenous CO₂ may have been attenuated during the present conditions because of loss of CO₂ into the aCSF.

Oxygen debt

Reoxygenation of anoxic slices caused an overshoot in heat production. A similar phenomenon has been observed in microcalorimetric experiments with mussels (*Mytilus edulis*) (Shick *et al.* 1986) and, with regard to body O₂ consumption, the goldfish shows an overshoot after anoxia although it does not accumulate lactate (Van den Thillart and Verbeek, 1991). The overshoot may reflect a restoration of phosphocreatine and glycogen levels. It is tempting to suggest that it also includes a compensatory increase in protein turnover, assuming that protein synthesis is depressed in anoxic slices, as it is in turtle hepatocytes (Land *et al.* 1993).

Lack of ethanol production

Although ethanol is the main metabolic end product in anoxic *Carassius* (Van Waarde *et al.* 1993), no ethanol was produced by the brain slices. This agrees with the results of previous studies showing that the ethanol-producing enzyme alcohol dehydrogenase (ADH) does not occur in crucian carp brain, while very high ADH activities are found in the skeletal muscles (Nilsson, 1988). Thus, the lactate produced by the brain has to be transported to the skeletal muscles before it is transformed into ethanol.

The glycolytic component in crucian carp brain slices

The value of 68 kJ mol⁻¹ of lactate presently obtained for glycolytic heat production (glucose to lactate) is strikingly close to the 70 kJ mol⁻¹ measured for the same reaction in mammals (Gnaiger and Kemp, 1990), although such estimates are likely to be influenced by temperature and by differences in extracellular buffering affecting the enthalpy of proton neutralisation (Gnaiger, 1983). Using the heat production value of 68 kJ mol⁻¹, it is possible to calculate the contribution of anaerobic glycolysis to the heat and ATP production in the slices. The amount of lactate produced during 20 h in oxygenated aCSF was 163±26 μmol g⁻¹. This corresponds to a heat production of 11.1±1.8 J g⁻¹. The heat produced by the slices during 20 h in oxygenated aCSF was 55.5±4.1 J g⁻¹, indicating that anaerobic glycolysis was responsible for 20±4% of the heat and 22±5% of the ATP produced by oxygenated crucian carp brain slices.

From the anoxic lactate production data, an average ATP turnover rate of 0.44 μmol g⁻¹ min⁻¹ during 20 h of anoxia at 12 °C can be calculated (1 mol of lactate yielding 1 mol of ATP). This value is half of that (0.88 μmol g⁻¹) obtained by Robin *et al.* (1979) for turtle brain slices kept at 21 °C, a discrepancy that can be fully explained by the temperature difference, Q₁₀ for brain energy metabolism in vertebrates being about 2.1 (Mink *et al.* 1981). Moreover, similar to the present results, Robin *et al.* (1979) found that 24% of the ATP produced during aerobic conditions came from anaerobic glycolysis, while the value they obtained for rat brain slices was 13%. This suggests that the glycolytic component in aerobic energy metabolism is higher in the anoxia-tolerant crucian carp and turtles than in mammals, although this

difference might be phylogenetic rather than being related to anoxia tolerance.

A 3.2±0.5-fold rise in lactate production was seen during anoxia, but the changes in glucose concentration in the aCSF were too small to be quantified. It is possible to estimate the magnitude of the increase in glucose consumption (Pasteur effect). If we accept the following: (1) that 31% less ATP was produced during anoxia (i.e. 0.44 and 0.64 μmol ATP g⁻¹ min⁻¹ in N₂ and O₂, respectively), (2) that 22% of the ATP produced in the presence of O₂ was derived from anaerobic lactate production, and (3) that 1 mol of glucose yields 2 mol of ATP when broken down to lactate and 25 mol of ATP during complete oxidation (Hochachka and Somero, 1984), then the glucose consumption would be 0.090 μmol g⁻¹ min⁻¹ in the presence of O₂ (0.020 μmol g⁻¹ min⁻¹ aerobically + 0.070 μmol g⁻¹ min⁻¹ anaerobically) and 0.22 μmol g⁻¹ min⁻¹ during anoxia. In other words, a 2.4-fold Pasteur effect. Although, this estimate will be affected by experimental errors, it seems highly unlikely that a Pasteur effect would be absent. The lack of a Pasteur effect would demand either a 70% metabolic depression, or that 70% of the ATP produced in the presence of O₂ be derived from anaerobic glycolysis, values falling far outside estimated errors in our data.

Brain energy consumption compared with other vertebrates

The present experiments suggest that the central nervous system (CNS) of *Carassius* is a very energy-demanding tissue. At the whole-body level, Van Waversveld *et al.* (1989) found that the heat production of normoxic *Carassius* (goldfish) kept at 20 °C was 1.40 J h⁻¹ g⁻¹. At 12 °C, we found the normoxic heat production of the brain slices to be 2.78 J h⁻¹ g⁻¹. Assuming that the metabolic rate of slices is half that *in situ* (Lipton and Whittingham, 1984), and a Q₁₀ for brain energy metabolism of 2.1 (Mink *et al.* 1981), then the heat production of the crucian carp telencephalon *in situ* would be 10.0 J h⁻¹ g⁻¹ at 20 °C. The CNS of a 10 g specimen of *Carassius* weighs about 100 mg. If we make the assumptions that the whole-body metabolic rates of both *Carassius* species are equal and that the metabolic rate of the telencephalon is representative for the entire CNS, then these calculations suggest (1) that the specific heat production rate of *Carassius* CNS is seven times higher than the whole-body heat production rate, and (2) that the CNS is responsible for 7% of the total energy consumption in this species. Mink *et al.* (1981) made a very similar estimate for the goldfish CNS (7.4%), although they lacked empirical data on metabolic rate in *Carassius* CNS. By comparison, the rat brain is responsible for 5.7% of the total energy consumption (Mink *et al.* 1981). Apparently, the genus *Carassius* has not sacrificed CNS function by having a small and energetically inexpensive CNS in order to facilitate anoxic survival.

In conclusion, adenylate phosphates and energy charge were maintained while heat production decreased during anoxia in telencephalic brain slices of crucian carp. The results show that this tissue has an intrinsic ability to tolerate anoxia and indicate

that its defence against anoxic damage involves metabolic depression combined with increased glycolytic ATP production.

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