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Depletion of high energy phosphates implicates post-exercise mortality in carp and trout; an *in vivo* ^{31}P-NMR study

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Abstract

As *in vivo* ^{31}P-Nuclear Magnetic Resonance spectroscopy is currently the state of the art method to measure continuously intracellular pH (pH_i) and energy status of muscle tissue, we used this method to study the recovery from exhaustive exercise. The biochemical changes during recovery are not well understood and it was suggested that post-exercise mortality could be caused by low pH_i; other studies however indicate that energy depletion might be more important. To analyse the mechanism of post-exercise recovery pH_i, ATP, P_i, and PCr must be measured at the same time, which is possible using *in vivo* ^{31}P-NMR. Common carp and rainbow trout of about 100 g were exercised to exhaustion in a swim tunnel. After swimming 10 h at 1.5 body lengths (BL)/s (aerobic control), 50% of the fish were forced to swim at 6 BL/s until exhaustion. Recovery of energy rich phosphates was found to be faster in carp (1.2–1.9 h) than in trout (1.5–2.3 h). The same applied for the recovery from acidosis, which took 1.75 h in carp and 5.75 h in trout. In parallel experiments the energy phosphates and lactate levels were measured in liver, red muscle, and white muscle. Exhaustion caused a significant drop in the energy status of red and white muscle tissue of trout and carp (corroborates NMR data), while no change at all was observed in liver tissue. The lactate levels were increased in the muscle but not in liver and blood. While all experimental animals looked healthy after exhaustion, 40–50% of the carp as well as trout died during the recovery phase. The energy status of those individuals measured by ^{31}P-NMR was much lower than that of the survivors, while in contrast there was no difference in pH_i. Thus, it appears that not acidosis but depletion of high energy phosphates disabled muscle function and therefore may have been the cause of death of the non-survivors.

Keywords: ^{31}P-NMR; Exercise; White muscle; Liver; Trout; Carp; Anaerobic metabolism; Creatine kinase; Collapse; Burst activity; Energy metabolism; Energy status; Intracellular pH; Acidosis

1. Introduction

Exhaustive exercise in fish is primarily powered by white anaerobic musculature. Burst-type exercise in fish generally involves the use of three endogenous fuels stored within the white muscle: glycogen, ATP and phosphocreatine (PCr). Energy is in the early stages of activity mainly derived from the breakdown of ATP and PCr. A similar amount of anaerobic energy can be produced by glycolysis, resulting in the accumulation of lactate. The net conversion of glycogen to lactate causes tissue acidosis, which is however, modulated by the alkaline reaction of creatinephosphate (PCr) and the acidic reaction of ATP hydrolysis (van den Thillart and van Waarde, 1996). Peak levels of lactate and inorganic phosphate are an indication for anaerobically produced energy during burst exercise. Following a bout of exhaustive exercise the recovery of PCr is generally quite rapid, usually within 1 h post-exercise. Unlike the recovery of PCr however, the removal of muscle lactate via the monocarboxylate transporter, oxidation and resynthesis of glycogen is in fish much slower (Kieffer, 2000).

After exercise till exhaustion significant mortality of fish occurs which may be as high as 40% (Wood et al., 1983). This

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high mortality rate during post-exercise is not immediate but happens several hours after collapse suggesting that it is mainly due to an impaired capacity to recover. It certainly must be due to extreme physiological disturbances. Major metabolic changes under those conditions are: a) depletion of glycogen and high energy phosphates, b) accumulation of lactate and concomitant metabolic acidosis, c) ionic and osmotic imbalance, and d) a surge in plasma catecholamines and cortisol. Wood et al. (1983) suggested that of these factors the intracellular acidosis of the white muscle compartment might be the proximate cause of post-exercise mortality in fish. Although acidosis inhibits many cellular processes, several studies suggest that a change in membrane potential might be the major factor leading to cell death (Buck et al., 1993). This change is set in motion by a low ATP level, which impairs ion-fluxes and particularly results in uncontrolled high Ca^{++}-influx.

In previous *in vivo* ^{31}P-NMR studies we observed that when energy stores were severely depleted during anaerobiosis, i.e. when [ATP] <30% of normoxic values, the experimental fish could not recover (van den Thillart et al., 1989b; van Ginneken et al., 1995). Based on that observation we hypothesized that the proximate cause of death of fish after extreme exercise may be depletion of energy stores. This is in contrast with the suggestion of Wood et al. (1983) who stated that the proximate cause is extreme intracellular acidosis.

A first step to solve this question is to measure the dynamics of both pH_i and energy status in the recovering white muscle. A most useful technique for this is ^{31}P-NMR, as it has the advantage that it is non-invasive and non-destructive. In addition, artifacts caused by tissue sampling and extraction are eliminated and semi continuous monitoring is possible.

In vivo ^{31}P-NMR has been applied to study the effects of environmental hypoxia and anoxia on energy metabolism in fish (van den Thillart et al., 1989a,b, 1990; van den Thillart and van Waarde, 1993, 1996; van Waarde et al., 1990, 1991; van Ginneken et al., 1995, 1996, 1999).

In the present study we applied the same method to study the recovery from exhaustive exercise by rainbow trout, (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). With the use of swim tunnels, we exposed carp and trout to a controlled exhaustive swimming protocol. In order to make a clear distinction between the aerobic and anaerobic type of swimming, we let the fish swim overnight at low speed. The combination of swim tunnel exercise followed by *in vivo* ^{31}P-NMR-measurements provided new *in vivo* data on post-exercise recovery of fish muscle.

2. Materials and methods

2.1. Animals and handling

The experiments were performed with rainbow trout, *Oncorhynchus mykiss* (de Keyzerberg — Blitterwijk, The Netherlands) and common carp, *Cyprinus carpio*, (Agricultural University Wageningen, The Netherlands). The animals were kept in the laboratory in local tap water for at least 1 month at 18 °C, fed daily with Trouvit pellets (Trouw, Putten, The Netherlands), and acclimated to a light–dark cycle of 14–10 h.

2.2. Swim trials

The day before the onset of the experiment, the fish were anaesthetized with 3-aminobenzoate ethyl ester methanesulphonate (MS-222, Sigma, St. Louis, MO, USA) at a final concentration of 100 ppm and placed in a large Blazka swim tunnel as described by van den Thillart et al. (2004). The relation between rotor speed and water flow was determined by Laser-Doppler technique at the Hydraulics Laboratory TU, Delft (The Netherlands), the set up was calibrated and almost linear between 0.2 and 1.0 m/s.

In order to reduce stress incurred by forced swimming, we used (overnight) 10 h swimming at 1.5 BL/s as a starting condition. This is a rather low speed which both species can endure for long periods. At the end of this standardized low exercise load, the control animals (CO) were anaesthetized and placed in the flow-through cell for the NMR-measurements or were sacrificed and sampled. A second group was forced to exhaustion after the 10-h period. This was reached by a stepwise increase of the speed to 6 BL/s, which was maintained until collapse. Starting at 1.5 BL/s the speed was increased every 30 min with 0.5 BL/s. After failure the fish were anaesthetized with 100 ppm MS-222, and placed in the NMR flow-through cell or killed for tissue sampling. In this protocol trout swam at 6 BL/s for 30–120 min before collapse, carp swam at 6 BL/s for 20–30 min.

2.3. ^{31}P-NMR-measurements

After the swim trial the anaesthetized fish were placed in a Perspex flow-through cell, which fitted in a modified Bruker bioprobe as described before (van den Thillart et al., 1989a). The fish was immobilized with an inflatable plastic bag filled with water and pressed with the left body side against the flat window of the flow-through cell. At the same time a tube was placed in the mouth of the fish and the gills were irrigated with a constant water flow of 170 mL min^{-1}. The temperature of the experimental set up was controlled by a water bath at 18 °C, while the total experimental set up was placed in a thermostatted room. The animals awoke within 5 min after transfer and stayed quiet in the dark of the magnet, which could be deduced from NMR spectra. Placing the fish in the flow-through cell took approximately 3 min. Optimizing the NMR signal by shimming and tuning took less than 10 min by an automatic routine.

In vivo ^{31}P-NMR spectra of the lateral musculature were obtained with a 9.4 T Bruker MSL-400 NMR spectrometer. The signal was picked up with an 18-mm surface coil, double-tuned to hydrogen (400 MHz) and phosphorus (162 MHz) frequencies. The coil was placed about 2 cm behind the operculum above the dorsal musculature and picked up a signal of the myotomes through the window of the cell. Shimming on the water signal optimized the homogeneity of the stationary magnetic field (B_o). The field homogeneity was considered adequate when the width of the H_2O peak was ≤0.25 ppm. Under optimal conditions shimming to 0.15 ppm was possible (60 Hz). ^{31}P NMR spectra (8192 data points) were accumulated over a period of 10 min and consisted each of 136 individual scans, using a pulse width of 60° (in the sensitive volume, the area within *ca.*