Control of energy metabolism in fish white muscle

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DRIEDZIC, WILLIAM R., AND PETER W. HOCHACHKA. Control of energy metabolism in fish white muscle. Am. J. Physiol. 230(3): 579-582. 1976. — Concentrations of key metabolites were determined in carp white muscle before exercise and after maximal activity. It was found that the concentration of ATP decreases by about 65%, ADP decreases slightly, and AMP remains low and unchanged. Consequently, the level of the free adenylate pool decreases. Simultaneously there is an increase in the concentration of IMP and NH₄⁺. The increase in IMP level and the decrease in adenylate pool are essentially in 1:1 stoichiometry, a result showing that the adenylate pool is decreased by the reaction catalyzed by 5'-AMP deaminase (EC 3.5.4.6). During exercise there is an increase in levels of glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-di-phosphate that, along with the decrease in ATP levels, can account for the increase in glycolytic flux by activation of phosphofructokinase and pyruvate kinase.

Adenylate pool; anaerobic metabolism; exercise; glycolysis

THE MYOTOMAL MUSCLE OF FISH is composed of red and white fibers superficially similar to those of mammals. In the carp (Cyprinus carpio) the white fibers represent about 90% of the muscle mass and by visual appearance alone may be dissected free from the adjacent red fibers. Although individual fibers within the white muscle mass of carp may differ in diameter (4), these fibers appear to be homogenous in terms of energy-generating properties (6, 10, 22). Thus the carp is an ideal species in which to study white muscle metabolism. On the basis of hemoglobin, myoglobin, and mitochondrial content, vascular supply, and enzymatic properties, the red muscle is considered to have a metabolism that functions primarily aerobically, whereas the white muscle is highly dependent on anaerobic means of energy generation (see 8). In fact, the major role of white muscle is to act as a powerful but temporally limited energy source for burst movement, such as required in capturing prey or avoiding predators (see 18).

There is a vast area of literature, recently reviewed by Bilinski (3), on the quantitative relationship between glycogen depletion and lactate accumulation in fish muscle during exercise. Sufficient for the present to reiterate the common finding: the greater the energetic demand placed on muscle, either by swimming longer at a given speed (above a certain level) or by swimming faster for the same length of time, the greater the depletion of glycogen and accumulation of lactate. Glycogen mobilization in fish white muscle is so rapid that during burst activity half of this fuel source may be depleted in 15 s with a concomitant production of lactate (28). The glycolytic pathway in fish muscle is similar to that described for other species, phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) are key regulatory enzymes and both are subject to allosteric regulation (11). Even though a wide range of metabolites have been shown to affect phosphofructokinase and pyruvate kinase in vitro, the mechanisms facilitating glycolytic flux in vivo, during burst activity, are unknown. Hence, a study was initiated of key metabolic regulators, particularly the free adenylate pool, before and after maximal activity.

The results of the present study show that after maximal activity there is a decrease in the total level of adenylates and increases in the concentration of NH₄⁺ and IMP in white muscle. Moreover, increases in the level of six carbon intermediates, along with alterations in the free adenylates, are consistent with increased glycolytic flux through activation of phosphofructokinase and pyruvate kinase.

MATERIALS AND METHODS

Animals. Carp (Cyprinus carpio), 12–15 cm in length, were obtained from a local pond. Animals were maintained at 12 ± 1°C in aerated running water under a simulated natural photoperiod and were fed frozen corn ad lib. Fish were exercised at 11°C in a swim tunnel; details of the apparatus and precautions to ensure adequate functioning have been described by Jones (13). Fish were introduced into the tunnel and forced to swim at 8.6 cm/s for 1 h. After the introductory phase the fish were subjected to 10-min periods of swimming at fixed velocities, after which the velocity was rapidly increased. The velocity increment was approximately 7 cm/s. The experiment was terminated when the fish could not remove itself from an electrified grid at the rear of the chamber. This usually occurred during the fourth speed increment. The average final velocity that the fish achieved, as empirically calculated by Brett (5), was 31 cm/s. Failure of a fish to meet the imposed velocity was not due to exhaustion of the white muscle, for after the experimental period an animal was still able to perform burst activity if forced to do so. Thus, in the present study there is no reason to believe that the white muscle was fatigued.

Preparation of tissue. Fish were removed from the holding tank or the swimming tunnel and immediately decapitated. A portion of white muscle weighing approximately 1 g was dissected from immediately below
the dorsal fin starting at the posterior margin and going anteriorly. The tissue sample was then frozen in liquid nitrogen. Time elapsed between removal of the animal from water and the freezing of tissue was about 5 s. The frozen tissue was powdered with a mortar and pestle that had been previously cooled and then the sample was placed in a 40-ml plastic centrifuge tube into which a Teflon pestle could fit snugly. The test tube contained an aliquot of cold HClO₄ (8% wt/vol) in 40% ethanol. The sample was mixed quickly with a glass rod and the amount of HClO₄ was taken up to 3.5 ml/g tissue. The tissue was homogenized for 2 min at high speed with a VirTis no. 23 mixer, during which time the test tube was maintained in a Dry Ice-ethanol bath. The homogenate was spun at 25,000g (4°C) for 10 min to precipitate protein. The supernatant solution was saved and the precipitate was resuspended in the same volume of 0.5 M potassium acetate; alanine by the addition of lactate dehydrogenase, pyruvate kinase, and adenylate kinase (1); fructose 6-phosphate and fructose 1,6-diphosphate by the sequential addition of glucose-6-phosphate dehydrogenase, phosphoglucose isomerase, and fructose-1,6-diphosphatase (24). Citrate was measured by the addition of malate dehydrogenase and citrate cleavage enzyme; malate by the addition of glutamic-oxalacetic transaminase and malate dehydrogenase; alanine by the addition of lactate dehydrogenase and glutamic-pyruvic transaminase; aspartate by the addition of malate dehydrogenase and glutamic-oxalacetic transaminase (20). In separate reaction mixtures NH₄⁺, lactate, and α-glycerophosphate were determined by use of glutamate dehydrogenase (15), lactate dehydrogenase (Sigma bulletin no. 826), and α-glycerophosphate dehydrogenase (20), respectively. Inosine monophosphate was assayed at 293 mμm by following the formation of uric acid after the addition of xanthic oxidase, nucleoside phosphorylase, and 5'-nucleotidase (7). All enzymes were purchased from Sigma. Results are expressed as micromoles of the specified substance per gram wet tissue. Water content of the tissue was determined to be 80%. The data are compared with the Student t test and a probability of less than 0.05 was considered to be significant.

**RESULTS**

The results of the present study are summarized in Table 1. The content of ATP decreases with activity. When the animals are exercised, ATP concentrations are reduced by about 65%. Levels of ADP also decrease a small but significant amount; however, AMP concentrations remain low and unchanged. Thus in this complex way the total free adenylate pool decreases during the exercise period. Concomitant with this decrease is an increase in IMP concentration. The increase in IMP level and the decrease in the adenylate pool are essentially in 1:1 stoichiometry, a result clearly showing that the adenylate pool is decreased by the conversion of AMP to IMP and NH₄⁺. The latter reaction is catalyzed by 5'-AMP deaminase (EC 3.5.4.6), which is known to occur in high levels in carp white muscle (J. I. A. Fields, personal communication). Preliminary studies show that at intermediate swimming speeds there is an intermediate decrease in the adenylate pool with a concomitant increase in IMP concentration. These data, however, are difficult to interpret since the degree of activity of individual fibers is unknown. For this reason, they are not discussed in this paper. It is interesting to note that, although NH₄⁺ concentration increases in working white muscle, the change is not as large as for IMP.

The energy charge, \([ATP] + 0.5 [ADP]/[ATP]\), is high in both groups of animals: 0.89 in the rested fish and 0.83 in the exercised group (Table 2). The apparent equilibrium constant of the adenylate kinase reaction is 0.3 in both the rested and exercised animals.

**TABLE 1. Metabolite concentrations in white muscle of resting and maximally exercised carp**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration, μmol/g fresh tissue</th>
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<tr>
<td>ATP</td>
<td>4.12 ± 0.18</td>
</tr>
<tr>
<td>ADP</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>AMP</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>IMP</td>
<td>1.38 ± 0.26</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>3.00 ± 0.30</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate</td>
<td>0.85 ± 0.08</td>
</tr>
<tr>
<td>Lactate</td>
<td>3.71 ± 0.17</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.62 ± 0.46</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>Malate</td>
<td>1.12 ± 0.28</td>
</tr>
<tr>
<td>α-Glycerophosphate</td>
<td>2.52 ± 0.60</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. At intermediate swimming velocities, intermediate values are generally obtained, but these are difficult to interpret because neither the number of white fibers recruited nor their degree of activation can be ascertained. * Statistically significant difference between groups (P < 0.05).

**TABLE 2. Total adenylate pool and energy charge in carp white muscle**

<table>
<thead>
<tr>
<th></th>
<th>Rested</th>
<th>Exercised</th>
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<tbody>
<tr>
<td>Adenylate pool</td>
<td>5.18 ± 0.18</td>
<td>2.68 ± 0.10*</td>
</tr>
<tr>
<td>Energy charge</td>
<td>0.89</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Adenylate values are means ± SE; n = 7. * Statistically significant difference between groups.
The concentrations of all of the glycolytic intermediates measured increase during activity. The greatest change occurs in lactate levels, which increase by about 10 \( \mu \text{mol/g} \). The mass-action ratio of the phosphofructokinase reaction is 1.82 in the rested fish and 2.78 in the exercised group. These values are displaced from the equilibrium constant of reaction by about 2 orders of magnitude (30) and support the concept of a regulatory role of phosphofructokinase in this tissue.

Of the two amino acids measured, aspartate levels decrease by a small but significant amount whereas there is a tendency for an increase in the level of alanine. As with alanine there is a tendency for \( \alpha \)-glycerophosphate levels to increase during activity. Of the two compounds measured associated with the Krebs cycle, malate concentration remained constant but citrate levels showed a tendency to decrease, although this was not statistically substantiated.

**DISCUSSION**

With respect to control of glycolysis per se, the situation in carp white muscle seems fairly clear. Increasing concentrations of glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and lactate during the exercise period indicate that, as expected, the glycolytic contribution to energy production is increased during high work rates. Activation of the two key regulatory enzymes of glycolysis, phosphofructokinase and pyruvate kinase, may be explained on the basis of the known kinetic properties of these enzymes. Thus substrate and product activation (9) of phosphofructokinase (by fructose 6-phosphate and fructose 1,6-diphosphate, respectively) with concomitant fructose 1,6-diphosphate feed-forward activation of pyruvate kinase, commonly observed in fish muscle pyruvate kinases (27), could readily account for the observed increase in glycolytic rate. Moreover, deinhibition of these two enzymes would be expected as a consequence of falling levels of ATP, a process facilitated by fructose 1,6-diphosphate (9, 27). Finally, further deinhibition of phosphofructokinase may occur due to decreasing levels of citrate (9). In these control characteristics, carp white muscle glycolysis appears to be similar to other more commonly studied systems [see Hochachka and Storey (12) for amplified discussion of this area]. Two minor inconsistencies with the literature deserve mention, however. First, it is evident from our data that the energy charge is essentially identical at both levels of muscle metabolism and muscle work. Although in vitro both phosphofructokinase (26) and pyruvate kinase (23) are stimulated by a decrease in this parameter, in vivo it is clear that energy charge plays only a modest role in sustaining the high glycolytic rates that support higher work rates. Second, there is no evidence whatever that AMP constitutes an uniquely important metabolite signal to muscle glycolysis, as suggested by Newsholme (21), for its concentration is similar at the two widely differing glycolytic rates. In contrast, if there is a single adenylate signal that is important to a sustained high level of glycolysis it presumably is ATP, since its overall concentration change is the greatest. As we argue below, however, in order to take advantage of this metabolic signal the organism must tolerate an overall reduction in the adenylate pool.

The 1:1 stoichiometric relationship between adenylate pool depletion and IMP accumulation, a result also obtained with cod muscle (14), clearly shows that the adenylate pool is reduced by the reaction catalyzed by 5'-AMP deaminase. The regulatory nature of this enzyme from carp white muscle has been well characterized (J H A. Fields, personal communication). The enzyme is activated by ADP \( K_a \approx 0.5 \text{ mM} \) and potentially inhibited by GTP \( K_i \approx 50 \mu \text{M} \). From the present study the enzyme appears to be controlled largely by the removal of GTP inhibition, because the guanine nucleotide pool is very low in fish muscle (14) and GTP is undetectable in muscle of fatigued fish (14). It is probable that, as the demands for high-energy phosphates increase during activity, the succinic thiokinase step of the Krebs cycle is not able to maintain GTP levels. These effects may be potentiated by falling concentrations of creatine phosphate, a known inhibitor of mammalian muscle 5'-AMP deaminase (25). Regardless, as 5'-AMP deaminase is deinhibited, IMP increases at the expense of AMP, which is in equilibrium with the other adenine nucleotides by the adenylic kinase reaction.

Lowenstein (19) has proposed that the 5'-AMP deaminase reaction is one step in a reaction span that is termed the purine nucleotide cycle. According to Lowenstein, IMP further reacts with GTP and aspartate to form adenylosuccinate. The adenylosuccinate in turn is converted to AMP plus fumarate. It has been shown in homogenates of mammalian skeletal muscle that the cycle functions in concert with glycolysis (29). The theory, however, predicts only a transient increase in IMP with general maintenance of the adenylate pool. Clearly the cycle per se does not operate during burst activity in carp white muscle since there is an accumulation of IMP. If the cycle were to become active only during recovery of the adenylate pool after exercise, the discrepancy between our results and those of Tornheim and Lowenstein (29) would be apparent rather than real.

It is interesting that the increase in \( \text{NH}_4^+ \) content in muscle during activity was less than the adenylate pool decrease or IMP increase. Probably this \( \text{NH}_4^+ \) was spilling out of muscle cells, as there are no nitrogen carriers in fish muscle comparable to glutamine in mammalian muscle. Since production of \( \text{NH}_4^+ \) under these conditions is probably totally anaerobic, our results provide an explanation for the observed phenomenon of anaerobic \( \text{NH}_4^+ \) production and excretion by intact fish (16).

We thank Mr. J. Kiceniuk both for collecting the animals used in this study and for exercising some of them. This work was supported by an operating grant from National Research Council of Canada to P.W.H. W. R. D. was the holder of a National Research Council of Canada Graduate Scholarship. Present address of W. R. Driedzic: Dept. of Physiology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pa. 17033.

Received for publication 17 March 1975.
REFERENCES


