Kinetics of Energy Metabolism in Skeletal Muscle during Ischemia

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Abstract : Rate equations of Lohmann's reaction, glycolysis and ATP hydrolysis were analyzed to introduce the relations between the rates of changes in phosphocreatine (PCr), ATP, inorganic phosphate (P_i) and proton (H^+) concentrations in skeletal muscle during ischemia. Kinetic analyses of the energy metabolism showed that the rate of PCr decrease is the same as that of P_i increase, when ATP remains unchanged, and that the rate of H^+ increase depends on the rate of glycolysis, although the source of H^+ is not glycolysis but ATP hydrolysis. Such kinetic analyses are useful for the assessment of the relations between the cross-linking PCr, ATP, P_i concentrations and intracellular pH, which are simultaneously measured with phosphorous magnetic resonance spectroscopy.

Key words : kinetics, energy metabolism, skeletal muscle, intracellular pH

Introduction

During ischemia, phosphocreatine (PCr) decreases, inorganic phosphate (P_i) increases, and intracellular pH (pH_i) decreases in skeletal muscle. These changes are well accepted, but relations between the rates of changes in the metabolite and proton (H^+) concentrations are still unsolved. Highly-contained PCr in skeletal muscle may contribute to the buffering action of the pH_i decrease during ischemia. Simultaneous measurements of PCr, ATP and P_i contents and pH_i of intact skeletal muscle are necessary to identify the buffering system. At present, phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is the most useful technique for the simultaneous and noninvasive measurements of the metabolites and pH_i. ^{31}P-MRS has been widely applied to the study of energy metabolism in intact functioning organs (Meyer *et al*., 1985; Bittl *et al*., 1987). We measured PCr, ATP, P_i and pH_i of rat skeletal muscle with *in vivo* ^{31}P-MRS during ischemia and hypoxia (Uchida *et al*., 1996), and those of perfused rat skeletal muscle with *in vitro* ^{31}P-MRS (Uchida *et al*., 1998).

^{31}P-MRS provides us time-dependent changes in PCr, ATP, P_i and H^+ contents, which are regulated with cross interactions. Kinetic analyses were applied to describe biochemical energy balance in skeletal muscle (Kushmerick, 1995). Kinetics are also important to estimate the phosphorylation potential (Veech *et al*., 1991). We have analyzed Lohmann's reaction, glycolysis and ATP hydrolysis, and shown that the rate of PCr decrease is the same as that of P_i increase with a constant ATP during ischemia. The rate of H^+ increase was found to depend on the rate of glycolysis, although the source of H^+ is not glycolysis but ATP hydrolysis.

Kinetic analyses

There are three main reactions in skeletal muscle during ischemia : Lohmann's reaction, glycolysis and ATP hydrolysis.

Lohmann's reaction $PCr^{2-} + ADP^{3-} + H^+ \rightarrow ATP^{4-} + Cr$ (1)

Glycolysis $1/2Glu + P_i^{2-} + ADP^{3-} \rightarrow LA^- + ATP^{4-} + H_2O$ (2)

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ATP hydrolysis $ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+$, (3)

where Cr is creatine, Glu is glucose, and LA^- is lactate ion. At physiological pH in cytosol, ADP and ATP are almost completely ionized and exist largely as the $MgADP^-$ and $MgATP^{2-}$ complexes. Putting the rate constants of the reactions (1), (2) and (3) as k_1, k_2 and k_3, respectively, we obtain the rates of changes in the metabolite and H^+ concentrations :

$$d[PCr^{2-}]/dt = -k_1[PCr^{2-}][ADP^{3-}][H^+] \quad (4)$$

$$d[ATP^{4-}]/dt = k_1[PCr^{2-}][ADP^{3-}][H^+] + k_2[Glu]^{1/2}[P_i^{2-}][ADP^{3-}] - k_3[ATP^{4-}] \quad (5)$$

$$d[H^+]/dt = k_3[ATP^{4-}] - k_1[PCr^{2-}][ADP^{3-}][H^+] \quad (6)$$

$$d[P_i^{2-}]/dt = k_3[ATP^{4-}] - k_2[Glu]^{1/2}[P_i^{2-}][ADP^{3-}] \quad , \quad (7)$$

with parentheses indicating concentrations of the metabolites and H^+. These rate equations contain three terms corresponding to the three rate constants :

$$A = k_1[PCr^{2-}][ADP^{3-}][H^+] \quad (8)$$

$$B = k_2[Glu]^{1/2}[P_i][ADP^{3-}] \quad (9)$$

$$C = k_3[ATP^{4-}] \quad . \quad (10)$$

Equations (4)—(7) can be written as follows with A, B and C.

$$d[PCr^{2-}]/dt = -A \quad (11)$$

$$d[ATP^{4-}]/dt = A + B - C \quad (12)$$

$$d[H^+]/dt = C - A \quad (13)$$

$$d[P_i^{2-}]/dt = C - B \quad . \quad (14)$$

If ATP content remains unchanged during ischemia,

$$d[ATP^{4-}]/dt = A + B - C = 0 \quad . \quad (15)$$

Therefore,

$$d[H^+]/dt = C - A = B \quad (16)$$

$$d[P_i^{2-}]/dt = C - B = A \quad . \quad (17)$$

From Eqs. (11) and (17), we can find that the rate of PCr decrease is identical to that of the P_i increase. If the reactions (1) and (3) are synchronized, ATP content is kept constant with the balance of input from PCr and output to P_i ($PCr \rightarrow ATP \rightarrow P_i$).

Equation (16) shows that the rate of the H^+ increase depends on B, or the rate of glycolysis (Eq. (2)). It should be noted that H^+ itself does not appear in Eq. (2). The source of proton is not glycolysis but ATP hydrolysis. Equation (2) can be obtained by summing up the sequential reactions of glycolysis (Kashiwaya *et al*. 1994). If the rate of the ATP hydrolysis is the same as that of Lohmann's reaction (A = C), the rate of the H^+ increase becomes zero from Eq. (16). Lohmann's reaction can remove H^+ originated from the ATP hydrolysis, while glycolysis cannot. pH_i decrease in skeletal muscle during ischemia is probably due to the alteration of ATP source from Lohmann's reaction to glycolysis. In our ^{31}P-MRS experiments with perfused rat biceps femoris (Uchida *et al*., 1998), pH_i did not decrease at the beginning of ischemia with sufficient PCr. With decreasing PCr, the main reaction for the ATP production should be switched from Lohmann's reaction to glycolysis. This turning point is likely to occur at the PCr/(PCr + P_i) ratio of about 0.6. Similar turning point of the ATP source was reported to appear during exercise (Sunoo *et al*., 1996).

Conclusion

In this report we neglect pH and temperature dependence of the rate constants. It is, however, possible to introduce essential relations between the reaction rates with simple kinetic analyses. The rate of the PCr decrease is shown to be the same as that of the P_i increase, when ATP remains unchanged during ischemia. The rate of the H^+ increase depends on the rate of glycolysis, although the source of H^+ is not glycolysis but ATP hydrolysis. Kinetics

of energy metabolism are useful to assess the relations between the rates of changes in PCr, P_i, ATP concentrations and pH_i, which are simultaneously measured with ^{31}P-MRS.

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