Inorganic Phosphate is not Limiting in Glycogenolytic Initiation

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Introduction

Glycogenolysis, consisting of glycogen degradation followed by glycolysis, is a fundamental metabolic pathway. It is believed that in anaerobic skeletal muscles, P-metabolites (P_i, AMP, and ADP) play a role in control of glycogenolysis. Inorganic phosphate (P_i) could have a limiting role in glycogenolysis since it is a substrate for glycogen degradation to glucose-1-P by glycogen phosphorylase a. In addition, P-metabolites could act as activators of glycogen phosphorylase and/or glycolysis. Evidence for this lies in the lack of correlation between glycogen phosphorylase a content and glycogenolysis, and in a correlation between P_i concentrations and the rate of glycogenolysis (1, 2, 3). Additional evidence comes from ^{31}P MRS studies demonstrating a delayed onset in glycogenolysis only when P-metabolite concentrations are low (4, 5). The results of these studies suggest that when P-metabolite concentrations are low, little to no glycogenolysis occurs; once P-metabolites accumulate, glycogenolysis may proceed. However, this study demonstrates delayed onset of glycogenolysis unaffected by P_i concentrations.

Methods

Gastrocnemius muscles were isolated from frogs (*Rana pipiens*). Muscles were kept in a oxygenated modified Ringer's solution (105 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 10 mM TRIS, pH=7.4, 4°C), then placed in Ringer's solution with 2.0 mM NaCN to inhibit oxidative metabolism 1 hr prior to data acquisition. Maximal stimulation of the muscles (≈23 mA/cm^2, 10 ms pulses, 1 Hz for 40 s) was done to produce twitches while within a GN300 spectrometer (GE-NMR, Fremont CA; Libra controller, Tecmag, Houston, TX). Five trains of twitches were used, separated by 3.5 min rest periods.

For one pulse ^{31}P MRS, the parameters were: 1.25 s TR, 4096 points, ±4000 Hz spectral width, and 45° flip angle (27 μs pulse width). Eight blocks of 10 s acquisitions were performed, each block consisting of 8 acquisitions. The twitches occurred during the second to fifth acquisition block. Spectral peak areas and position were determined by Lorentzian fitting. Quantification done using 6.2 mM of ATP as an internal standard. Partial saturation correction used T_1 values of 4.1, 2.1, 1.3, 0.9, 0.9 s for P_i, PCr, γATP, αATP, βATP respectively). pH was determined from the P_i peak position using pK_a = 6.81, = 3.27, = 5.63.

Data from 3 experiments were averaged together.

Results

Figure 1

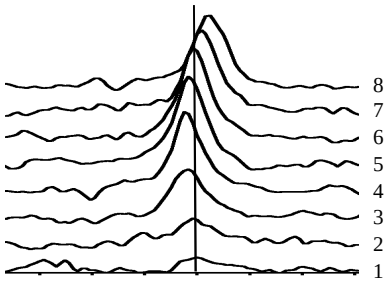

Figure 2

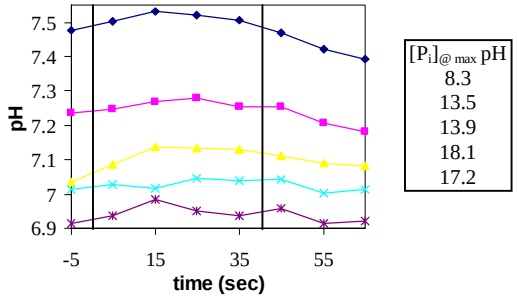

Figure 1. Stack plot of the P_i peak before (1), during (2-5), and after (6-8) the 1 Hz electrical stimulation. Initial alkalization (left shift) indicating PCr hydrolysis and subsequent acidification (right shift) when glycogenolysis dominates. Peak increases in size as PCr hydrolysis results in P_i accumulation.

Figure 2. The pH measured by P_i. Transient alkalinization occurs during each twitch train (between vertical lines). [P_i] at the peak pH for each twitch train in box.

Discussion

In skeletal muscles, the ratio of phosphorylase a to b does not correspond to the glycogenolytic rate (1, 2, 3). An additional mechanism of regulation could be via P_i, since a correlation between [P_i] and glycogenolytic rate has been observed (1, 2, 3). From ^{31}MRS comes evidence based on the observation of a transient alkalization in response to contractions only when P-metabolite concentrations are low (4, 5). The alkalization represents PCr hydrolysis, an H^+ consuming reaction, occurring with little to no production of lactic acid. It has been concluded that the glycogenolysis occurs when a threshold [P_i] was reached, giving P_i a limiting role in glycogen degradation (4).

Our findings conflict with the prior MRS evidence that P_i is limiting in glycogenolysis. A transient alkalization followed by acidification is observed in response to each twitch train (fig. 2). The pH peaked during the second or third 10 sec acquisition period each time, irrespective of [P_i]. The [P_i] had no effect of the duration of the transient alkalization. Thus P_i content does not explain the delayed onset of glycogenolysis, since the initiation of glycogenolysis shows no correlation with [P_i].

The failure of others to observe alkalization at higher [P_i] could be due to increases in buffering capacity. Buffering capacity increases due to P_i accumulation, thus decreasing the magnitude of the transient alkalization.. It is also likely that there is a small contribution of H^+ by glycogenolysis during the initial alkalization phase which is not large enough to eliminate the transient alkalization, but it could have a significant impact on the magnitude of the pH alkalization (6), making it more difficult to observe. In the experiments by Yamada *et al*, Lac^- accumulation within lengthy MRS acquisition times could be occurring and would obscure the transient alkalization.

The observation of a transient alkalization irrespective of [P_i] demonstrates that P_i does not limit the initiation of glycogenolysis.

Acknowledgements

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