Control of glycolysis in contracting skeletal muscle. I. Turning it on

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Crowther, Gregory J., Michael F. Carey, William F. Kemper, and Kevin E. Conley. Control of glycolysis in contracting skeletal muscle. I. Turning it on. Am J Physiol Endocrinol Metab 282: E67–E73, 2002.—Why does the onset of glycolytic flux in muscle lag the start of exercise? We tested the hypothesis that both elevated metabolite levels and muscle activity are required for flux to begin. Glycolytic flux was determined from changes in muscle pH, phosphocreatine concentration, and Pi concentration ([Pi]) as measured by 31P magnetic resonance spectroscopy. Eight subjects performed rapid ankle dorsiflexions to ~45% of maximal voluntary contraction force under ischemia at a rate of 1 contraction/s. Subjects completed two bouts of exercise separated by 1 min of ischemic rest. Glycolytic flux was activated by 27 s in the first bout, ceased during the ischemic rest period, and was activated more quickly in the second bout. Because the onset of ischemic rest. 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A delayed onset of glycolytic flux is, on the surface, consistent with the conventional feedback control model of the pathway. This model holds that the metabolites generated by muscle contraction, such as Pi, ADP, and AMP, increase flux by acting as substrates and allosteric activators of glycogenolytic and glycolytic enzymes (10). Evidence for the role of metabolites in turning on glycolysis is the finding that glycolytic flux does not begin until P concentration ([P]) is elevated in both resting and active isolated frog muscle (39, 40). This finding is in accord with the role of P as a substrate for glycogen phosphorylase (GP) and an allosteric activator of phosphofructokinase (PFK; see Ref. 10). Not consistent with the feedback control model is the finding that elevated metabolite levels are not sufficient to maintain glycolytic flux. Both human and animal studies have shown that the flux subsides at the end of exercise, even if metabolites are kept high (12, 13, 32, 37). Thus elevated metabolite levels may be necessary for the activation of glycolysis, but they alone are insufficient to maintain flux.

A control scheme that could reconcile these results is a “dual-control” model in which both metabolites and muscle contraction (specifically intracellular Ca2+) affect flux. Such dual control of flux occurs at the GP step of glycogenolysis. GP is converted to its more active a form through the action of Ca2+ (10). In addition, GP requires P as a substrate and therefore is activated by increases in [P] (33). Thus glycogenolysis is subject to dual control by intracellular Ca2+ and a metabolite. Can this same dual-control system account for the onset and maintenance of glycolytic flux?

This study tested the hypothesis that elevated metabolite concentrations, in addition to muscle activity, are needed for glycolytic flux to begin. Our experimental design varied the metabolite levels at the start of exercise to determine whether they affect the onset of glycolytic flux. Specifically, subjects performed two bouts of ischemic exercise, each consisting of voluntary 1-Hz contractions, separated by 1 min of ischemic rest. We predicted that the onset of glycolytic flux would be more rapid in the second exercise bout, when initial...
metabolite concentrations are high. A second purpose of the study was to determine whether glycogenolysis and glycolysis are turned on at different metabolite levels. We used 0.5-Hz exercise to slow the activation of these pathways, allowing us to collect data with lower time resolution and thus a better signal-to-noise ratio. For all experiments, we employed a voluntary ballistic exercise protocol that recruits all muscle fibers at low force levels (14, 15) and measured key intracellular metabolites and pH noninvasively using 31P magnetic resonance spectroscopy (MRS).

METHODS

Subjects

Eight adult men aged 25–61 yr and recruited from a population of normal volunteers were studied. The experimental protocols were approved by the Human Subjects Division of the University of Washington, and voluntary, written informed consent was obtained from each subject.

Experimental Setup and Data Acquisition

Each subject lay supine in the bore of a General Electric Signa 1.5 Tesla spectrometer. The right leg and foot were held in place with a plastic holder to which a strain gauge was attached. The strain gauge measured the force exerted by the ankle dorsiflexor muscles and was linked to a computer running LabView data acquisition software (National Instruments, Austin, TX). A 4.5 × 9.0-cm surface coil tuned to 25.9 MHz (the resonating frequency of phosphorus at 1.5 Tesla) was placed over the anterior compartment of the right leg. 31P magnetic resonance spectra of the ankle dorsiflexors were then acquired with the surface coil as previously reported (9). Briefly, the magnetic field homogeneity was optimized by off-resonance shimming on the proton peak of muscle water. The unfiltered PCR line width (full width at half-maximal height) was typically 3–5 Hz. A high-resolution control spectrum of the resting muscle was acquired under conditions of fully relaxed nuclear spins (interpulse delay: 16 s). Sequential spectra were then obtained under partially saturating conditions (interpulse delay: 1.5 s) throughout the experimental protocols described below. The spectrum for each time point consisted of four summed acquisitions taken over 6 s or eight summed acquisitions taken over 12 s. Rapidly acquired 6-s spectra typically had a signal-to-noise ratio of 90:1 for the PCR peak after line broadening.

Analysis of Spectra

Free-induction decays (FIDs) were summed, baseline corrected, zero filled, apodized with an exponential filter matched to the line width of the unfiltered PCR peak (3–5 Hz), and Fourier transformed into spectra. Fully relaxed spectra were manually phased, baseline fixed, and analyzed with the program “MacFID” (Tecmag, Houston, TX) to determine the area of each phosphorus peak. PCR-to-ATP and Pi-to-ATP ratios were determined from the relative areas of the appropriate peaks; the ATP peak area was taken to be the average of the areas of the α-, β-, and γ-ATP peaks. PCR peaks of partially saturated spectra were analyzed with the “Fit-to-Standard” program (21), whereas the P1 and phosphomonoester (PME) peaks were analyzed with “MRUI” (38) because their line shapes changed during exercise. Absolute PCR concentration ([PCr]) and [P1] were then calculated using the PCR-to-ATP and Pi-to-ATP ratios of the fully relaxed spectra and assuming the muscle ATP concentration to be 8.2 mM (20). Quantification of the PME peak was done similarly, except that the resting PME concentration ([PME]) was too small to be measured accurately by our methods and was assumed to be 1 mM on the basis of muscle biopsy data (5, 22, 36). Most PME generated during exercise are hexose phosphates (HP), i.e., glycolytic intermediates such as glucose 6-phosphate (G-6-P) and fructose 6-phosphate (5, 19, 22); therefore, changes in [PME] were considered equivalent to changes in [HP]. Free ADP concentration ([ADP]) and AMP concentration ([AMP]) were calculated assuming the creatine kinase and adenylate kinase reactions to be at equilibrium (28), with adjustments made for pH and Mg2+ concentration (18). The chemical shift of the P1 peak relative to PCR was used to calculate muscle pH (37).

Voluntary Exercise

Subjects exercised by performing voluntary isometric ballistic dorsiflexions against the resistance of the plastic foot holder. Subjects used a metronome to maintain the desired contraction frequency of 0.5 or 1 Hz (see below) and used visual feedback from a light-emitting diode display to achieve the desired peak force. For each contraction, subjects reached the target force as quickly as possible and then relaxed immediately. Rapid contractions of this type, termed “ballistic contractions” by Desmedt and Godaux (14, 15), have been found to recruit all the muscle fibers of the tibialis anterior when >25% of maximal voluntary contraction (MVC) force is generated (14). To ensure complete recruitment, our subjects reached ~45% of MVC force with each contraction.

Experimental Protocol: 1-Hz Exercise

The 1-Hz exercise trials were designed to test the hypothesis that elevated metabolite concentrations are necessary for the activation of glycolysis. The details are summarized in Table 1 and below.

Table 1. Experimental protocol for 1-Hz exercise trials

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aerobic rest</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>Ischemic rest</td>
<td>300</td>
</tr>
<tr>
<td>C</td>
<td>Ischemic exercise (bout 1)</td>
<td>12, 24</td>
</tr>
<tr>
<td>D</td>
<td>Postexercise ischemia</td>
<td>60</td>
</tr>
<tr>
<td>E</td>
<td>Ischemic exercise (bout 2)</td>
<td>42</td>
</tr>
</tbody>
</table>
Step D: Postexercise ischemia (60 s), stopping glycolytic flux. We wanted to make sure that glycolytic flux dropped to basal levels before the start of bout 2 (step E) and used changes in pH, [PCr], and [Pi] to quantify this flux.

Step E: Bout 2 of ischemic exercise (42 s), determining the onset of glycolytic flux when metabolites are high. Because of muscle activity in the first exercise bout, initial metabolite concentrations were elevated in this second bout. We therefore quantified glycolytic flux during bout 2 to test the hypothesis that the onset of flux is more rapid when metabolite concentrations are high (i.e., during bout 2) than when they are low (i.e., during bout 1).

Each subject completed steps A-E at least four times. The following two parameters varied among trials: 1) the duration of step C (the first exercise bout) ranged from 12 to 60 s, and 2) step E was omitted in trials where step C lasted 60 s to avoid fatigue in subsequent bouts. Thus each subject completed four different trials, each consisting of alternating periods of ischemic rest and ischemic exercise. The order of the trials was randomized. Subjects recovered aerobically for >500 s between trials, which was sufficient for PCr, Pi, and pH to return to baseline levels.

Experimental Protocol: 0.5-Hz Exercise

A lower contraction frequency (0.5 Hz) was used to compare the onset of glycolytic flux with the onset of glycogenolytic flux. The 0.5-Hz trial consisted of 60 s of aerobic rest, 300 s of ischemic rest, and 84 s of ischemic voluntary contractions. We quantified glycolytic flux and glycogenolytic flux (see below) to determine whether these fluxes are turned on at different metabolite levels.

Calculation of Glycolytic Flux

Glycolytic flux under ischemic conditions was quantified as glycolytic H+ production, since the lactic acid produced by glycolysis cannot be removed from the muscle by the circulatory system or be oxidized to CO2. Glycolytic H+ production was calculated from changes in muscle pH, [PCr], and [Pi] as previously described (see Fig. 3 in Ref. 6). In brief, H+ generation by the glycolytic pathway (Hglycol) equals the observed change in H+ concentration plus the H+ consumed in the breakdown of PCr.

\[
\Delta H_{\text{glycol}}^+ = \Delta pH \times \beta_{\text{bt}} + (-\gamma) \times \Delta [\text{PCr}]
\]

where \(\Delta pH\) is the change in muscle pH, \(\beta_{\text{bt}}\) is the total muscle buffer capacity (which includes buffering due to Pi; see Ref. 6), \(\gamma\) is the proton stoichiometric coefficient of PCr hydrolysis (27), and \(\Delta [\text{PCr}]\) is the change in [PCr]. Glycolytic H+ production is reported in Figs. 3 and 4 as “glycolytic H+ accumulation,” i.e., total (cumulative) H+ glycolytic production from the beginning of exercise to the time of each data point.

Glycolytic flux in the 0.5-Hz trials was calculated as glycolytic G-6-P use (\(\Delta G-6-P_{\text{glycol}}\)) to enable a direct comparison of glycolytic flux and glycogenolytic flux. This calculation reflects the stoichiometry that two protons (and 2 lactate molecules) are produced for each molecule of G-6-P catalyzed.

\[
\Delta G-6-P_{\text{glycol}} = \Delta H_{\text{glycol}}^+/2
\]

Calculation of Glycogenolytic Flux

Glycogenolysis is fueled almost exclusively by glycogenolysis in active ischemic muscle, as evident in the stoichiometry that 1.5 ATP are produced for every H+ generated (whereas the breakdown of glucose taken up from the blood results in a 1:1 ratio; see Ref 6 and erratum therein). Thus, for our experiments, the total glycogenolytic flux equals the glycolytic flux plus any additional glycogenolytic flux that has not passed through the glycolytic pathway. This latter component of the flux is represented by the build-up of HP, primarily as G-6-P (5). Hence, glycogenolytic G-6-P production (G-6-Pglycol) may be calculated as

\[
\Delta G-6-P_{\text{glycol}} = \Delta G-6-P_{\text{glycol}} + \Delta [\text{HP}]
\]

where \(\Delta [\text{HP}]\) represents the change in [HP].

Statistics

Reported values are means ± SE. Glycolytic fluxes were compared with zero by use of one-tailed t-tests, with \(\alpha\)-levels adjusted for multiple comparisons by means of a sequential Bonferroni correction (35). The first data point in each exercise bout to be significantly greater than zero (i.e., baseline) was considered to represent the onset of flux.

RESULTS

Ballistic Exercise

In studying voluntary contractions, we wanted to ensure that our measurements would not be confounded by incomplete muscle fiber recruitment. Desmedt and Godaux (14, 15) found that recruitment of all fibers is achieved in submaximal voluntary contractions of the tibialis anterior and two other muscles when the peak force (1) exceeds 25–35% of MVC force and (2) is reached as rapidly as possible (within 100–150 ms of the start of the contraction). To make sure that all dorsiflexor muscle fibers were active during exercise, we trained our subjects to exert ~45% of MVC force with typical rise times of 100–200 ms (Fig. 1), thus satisfying both criteria.

31P MRS Measurements Before, During, and After Exercise

Resting PCr-to-ATP and Pi-to-ATP ratios were 4.14 ± 0.13 and 0.45 ± 0.02, respectively, consistent with previously reported values (8).

Figure 2 shows 31P MRS summary data for one of the four 1-Hz exercise trials, in which ischemic rest periods alternated with ischemic exercise. During exercise bout...
1, there was a drop in [PCr] and a rise in [Pi], [ADP], [AMP], and pH. The rise in pH was the result of H⁺ consumption by PCr breakdown in the creatine kinase reaction. During ischemic rest, little change was apparent in metabolite levels or pH once exercise stopped. During exercise bout 2, there was a further drop in [PCr] and a rise in [Pi], [ADP], and [AMP], and a decline in pH occurred when exercise was resumed. Changes in [PCr], [Pi], and [HP] (data not shown) were stoichiometric throughout steps B-E. PCr, phosphocreatine. Data shown are mean values (n = 8); error bars are omitted for clarity.

Activating Glycolysis

The purpose of our 1-Hz experiments was to test whether the accumulation of metabolites (Pi, ADP, and/or AMP) is responsible for the onset of glycolytic flux. Our test consisted of determining whether this onset is accelerated when previous exercise has driven up metabolite levels. A period of ischemic rest was interposed between exercise bouts to allow short-lived contraction-related signals such as Ca²⁺ to dissipate while maintaining metabolite levels. A more rapid onset of glycolytic flux in exercise bout 2 would indicate that this onset is governed by a signal persisting between bouts (e.g., metabolite concentrations) rather than a short-lived contraction-linked signal (e.g., Ca²⁺).

Figure 3 shows glycolytic H⁺ production in each of the four 1-Hz exercise trials. Glycolytic H⁺ production began by -27 s (5 spectra) in a single bout of continuous exercise lasting 60 s (Fig. 3A). This onset occurred after three spectra in a bout preceded by a 12-s bout (Fig. 3B) and after one or two spectra in bouts preceded by 24- or 42-s bouts (Fig. 3, C and D). Because the duration of prior exercise increased (from A to D in Fig. 3), the onset of glycolytic H⁺ production occurred earlier. This finding that the onset time was reduced by prior exercise means that a short-lived signal related to contraction per se (e.g., Ca²⁺) is not solely responsible for the onset of glycolytic flux. However, the importance of contractile activity is seen in the fact that there was essentially no flux during the ischemic rest periods between bouts.

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To test the hypothesis that metabolite accumulation governs the activation of glycolysis, glycolytic H⁺ production is replotted as a function of contraction number (and therefore total exercise time) in Fig. 4. Glycolytic activation occurred by ~27 contractions (or ~27 s), regardless of whether these contractions were performed within the first exercise bout or were divided between the first and second bouts. Therefore, each contraction in the first exercise bout reduced the number of contractions needed to turn on glycolysis in the second bout. Table 2 lists the [Pi], [ADP], and [AMP] at rest and after 27 contractions for the four exercise trials, each of which drove the metabolite concentrations to similar levels. Taken together, Fig. 4 and Table 2 indicate that the onset of glycolytic flux coincides with elevated metabolite concentrations, suggesting that one or more of these metabolites may be involved in the activation of glycolysis.

**Onset of Glycolysis vs. Glycogenolysis**

We determined whether the onset time of glycolytic flux differed from that of glycogenolytic flux using 0.5-Hz exercise. The lower contraction frequency of 0.5 Hz permitted the summing of signals over 12-s periods, thus facilitating quantification of the low signal-to-noise HP peak. Figure 5 shows that HP levels increased 18 s into exercise, whereas significant glycolytic G-6-P use did not occur until 54 s. Thus HP levels were increased three spectra and 36 s before the apparent onset of glycolytic flux. In addition, we found that glycogenolytic flux and glycolytic flux began at distinct metabolite levels: [Pi] was 10.4 ± 0.8 mM at the onset of glycogenolytic flux, whereas significant glycolytic flux did not occur until [Pi] reached 18.0 ± 1.9 mM.

**DISCUSSION**

This study shows that elevated metabolite concentrations are critical to the activation of glycolytic flux in contracting muscle. The key role of metabolites is evident in the finding that elevated metabolite concentrations reduce the delay in the onset of flux. Furthermore, glycolytic flux and glycogenolytic flux turn on at distinct metabolite levels, suggesting that these pathways may be controlled separately.

**Activation of Glycolysis**

GP activity is responsive to both muscle activity (i.e., Ca²⁺) and metabolites (i.e., Pi and AMP; see Ref. 10). Similarly, both contraction- and metabolite-related signals appear to govern the activation of glycolysis. The importance of muscle contraction is apparent in the result that glycolytic H⁺ production ceases once exercise ends, even though metabolite levels remain high (Fig. 2). This finding in earlier studies (32, 37) has led to the conclusion that muscle activity (perhaps Ca²⁺ in specific) is required to sustain high glycolytic fluxes (6). The more rapid onset of glycolytic flux at high metabolite levels in this study (Fig. 3) demonstrates that metabolites are also important in the control of this flux. Expressing glycolytic H⁺ production as a function of contraction number reveals that the onset of flux occurred after ~27 contractions in each trial (Fig. 4), by which time Pₐ, ADP, and AMP had risen far above their basal levels (Table 2). The relatively wide ranges listed in Table 2 reflect the fact that metabolite concentrations are critical to the activation of glycolysis.
centrations change rapidly from spectrum to spectrum during 1-Hz exercise; nevertheless, it is clear that the onset of glycolytic flux is associated with elevated metabolite levels. These results suggest that accumulation of one or more metabolites, in addition to muscle activity, is necessary for the onset of glycolytic flux.

Glycogenolysis vs. Glycolysis

Glycolytic and glycogenolytic fluxes begin at different times (Fig. 5) and at different metabolite levels during 0.5-Hz exercise. We compared the two fluxes to determine whether the onset of glycolytic flux might be a mass-action response to the onset of glycogenolytic flux. In theory, this is plausible because glycogenolysis supplies glycolysis with most of its substrate (G-6-P) during the first few minutes of exercise (16, 23). However, a disparity between the onset of glycogenolytic flux and the onset of glycolytic flux is evident in Fig. 5. [HP] were elevated within 18 s of the start of 0.5-Hz exercise, whereas glycolytic flux was slower to rise and did not reach statistical significance until 54 s into exercise. Thus the glycolytic production of G-6-P does not appear sufficient to cause the onset of glycolytic flux, although it is possible that a certain “threshold” level of G-6-P is required for glycolytic flux to begin. In any case, our results coupled with the fact that glycogenolytic flux often exceeds glycolytic flux (6, 31) suggest that the two pathways differ in their respective sensitivities to the signals that control flux.

Sites and Mechanisms of Activation

Two key enzymes that affect glycolytic and glycogenolytic flux and that are stimulated by metabolites in vitro are GP and PFK. Can our current knowledge of the control of these enzymes explain our findings? A mechanism by which elevated metabolites may promote the onset of glycogenolytic flux has already been established. Pi is a substrate for GP, and at AMP levels ≤0.01 mM (as seen in Table 2), the in vitro Michaelis-Menten constant (Km) of GP for Pi is 20–27 mM (3, 4, 34). If the in vivo Km is similar to that measured in vitro, increases in [Pi] to ~10 mM (such as those reported here) should increase GP activity and glycogenolytic flux. The importance of Pi as a substrate for phosphoglycerate kinase and pyruvate kinase, whereas Pi is a substrate for glyceraldehyde-3-phosphate dehydrogenase and has been reported to relieve ATP inhibition of this enzyme in vitro (29, 30). Thus it is possible that a metabolite-induced activation of multiple glycolytic steps occurs via the elevation of substrates and allosteric activators of glycolytic enzymes. Moreover, it is now clear that a contraction-related signal such as Ca2+ is also important in the control of glycolytic flux (6, 32), yet the exact mechanistic basis of this control likewise remains uncertain (12).

In summary, we have examined the mechanisms controlling the activation of glycolysis in contracting human muscle. Our results show that the onset of glycolytic flux does not simply correspond to the onset of glycogenolytic flux but does require both muscle activation and elevated metabolite concentrations. It is not yet clear which metabolites are most important, how they promote flux, or which enzymes are involved. Nonetheless, our results emphasize the importance of both muscle contraction and metabolite levels in the initiation and maintenance of glycolytic flux.

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Portions of this work have been previously published in abstract form (11).

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