Hypoxia causes glycogenolysis without an increase in percent phosphorylase in rat skeletal muscle

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Ren, Jian-Ming, Eric A. Gulve, Gregory D. Cartee, and John O. Holloszy. Hypoxia causes glycogenolysis without an increase in percent phosphorylase in rat skeletal muscle. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1086–E1091, 1992.—Stimulation of skeletal muscle to contract activates phosphorylase b to a conversion and glycogenolysis. Despite reversal of the increase in percentage of phosphorylase a after a few minutes, continued glycogen breakdown can occur during strenuous exercise. Hypoxia causes sustained glycogenolysis in skeletal muscle without an increase in percentage of phosphorylase a. We used this model to obtain insights regarding how glycogenolysis is mediated in the absence of an increase in percentage of phosphorylase a. Hypoxia caused a 70% decrease in glycogen in epitrochlearis muscles during an 80-min incubation despite no increase in percentage of phosphorylase a above the basal level of ~10%. Muscle P_i concentration increased from 3.8 to 8.6 μmol/g muscle after 5 min and 15.7 μmol/g after 20 min. AMP concentration doubled, attaining a steady state of 0.29 μmol/g in 5 min. Incubation of oxygenated muscles with 0.1 μM epinephrine induced an approximately sixfold increase in percentage of phosphorylase a but resulted in minimal glycogenolysis. Muscle P_i concentration was not altered by epinephrine. Despite no increase in percentage of phosphorylase a, hypoxia resulted in a fivefold greater depletions of glycogen over 20 min than did epinephrine. To evaluate the role of phosphorylase b, muscles were loaded with 2-deoxyglucose 6-phosphate, which inhibits phosphorylase b. The rate of glycogenolysis during 60 min of hypoxia was reduced by only ~14% in 2-deoxyglucose 6-phosphate-loaded muscles. These findings suggest that 1) there is sufficient phosphorylase a in unstimulated muscle to mediate rapid glycogenolysis; 2) unavailability of Pi prevents net glycogenolysis in resting oxygenated muscle; 3) an increase in P_i can cause glycogenolysis in muscle in the absence of an increase in percentage of phosphorylase a; and 4) an increase in AMP might also be involved in causing glycogenolysis through phosphorylase a activation.

Adenine monophosphate; 2-deoxyglucose 6-phosphate; epinephrine; epitrochlearis muscle; inorganic phosphate

GLYCOGENOLYSIS is mediated by the enzyme phosphorylase, which catalyzes the phosphorolysis of the α-1,4-linked glucose residues in glycogen. The regulation of phosphorylase activity has been under intense study for roughly 50 years, beginning with the studies of the Coris and their co-workers (10–12). The picture that has emerged is of a complex network of interacting enzymes and cofactors that determine phosphorylase activity (6, 7, 10, 16, 19, 20, 22, 23, 34). Phosphorylase exists in two molecular forms. Phosphorylase b is inactive in the absence of AMP, whereas phosphorylase a is active in the absence of AMP or other effectors (13, 20, 23). Phosphorylase b is converted to the a form by phosphorylase kinase, which also exists in a dephosphorylated, less active b form that is converted to the active a form by kinase A in response to an increase in adenosine 3',5'-cyclic monophosphate (cAMP) (6, 16, 19, 20, 23). Phosphorylase kinase activity is Ca^{2+} dependent. Phosphorylase kinase a is active at the Ca^{2+} concentration found in resting muscle, whereas the b form is not active in resting muscle but becomes active at the cytosolic Ca^{2+} concentrations attained in contracting muscle (3, 7, 20, 23).

Although the enzymology of phosphorylase activation and deactivation has been elucidated in considerable detail, the regulation of glycogenolysis under physiological conditions is still not well understood. It is generally thought that glycogen breakdown in contracting skeletal muscle is mediated by the conversion of phosphorylase b to a that results from phosphorylase kinase activation by the Ca^{2+} released from the sarcoplasmic reticulum (3, 7, 20, 23). This mechanism is clearly involved in the large burst of glycogenolysis at the onset of contractile activity (4, 9, 35, 39). However, studies on muscle stimulated to contract in vitro (1, 9, 37, 39) and on muscles biopsied during exercise (4) have shown that the activation of phosphorylase is transient and that the proportion of phosphorylase in the a form (%phosphorylase a) decreases to, or below, the resting level after a few minutes despite continued contractile activity. Despite reversal of phosphorylase activation, muscle glycogen degradation, to the point of almost complete depletion, can occur during strenuous exercise (2, 9). Two possible explanations for this phenomenon have been suggested. One is that phosphorylase might be reactivated as a consequence of a β-adrenergic stimulation-mediated increase in cAMP (37, 39, 43). The other is that availability of P_i, not phosphorylase activity, limits glycogenolysis in resting muscle and that an increase in percentage of phosphorylase a is not needed for glycogenolysis to occur if cytosolic P_i concentration is increased sufficiently (4, 22).

Another stimulus that results in glycogenolysis in muscle is hypoxia. Morgan and Parmeggiani (31) have shown that hypoxia causes an increase in percentage of phosphorylase a in heart muscle. We had therefore assumed that glycogenolysis is also stimulated by an increase in percentage of phosphorylase a in skeletal muscle. However, during studies on the effect of hypoxia on glucose transport, we found no increase in percentage of phosphorylase a in skeletal muscle. Nevertheless, hypoxia causes rapid sustained glycogenolysis. In the present paper, we describe this phenomenon and report the results of experiments in which we used hypoxia to obtain insights regarding how glycogenolysis is mediated in the absence of an increase in percentage of phosphorylase a in skeletal muscle.

METHODS

Treatment of animals. Male specific pathogen-free Wistar rats weighing 100–130 g were obtained from Sasco (Omaha, NE) and fed Purina rat Chow and water ad libitum. The rats were anesthetized with pentobarbital sodium (5 mg/100 g body wt) given by intraperitoneal injection. The epitrochlearis muscles...
were then dissected out.

**Muscle incubation.** Epitrochlearis muscles were incubated for the indicated time periods in 2 ml of Krebs-Henseleit bicarbonate buffer (KHB) (25) containing 8 mM glucose and 32 mM mannitol in Erlenmeyer flasks, maintained at 35°C, in a Dubnoff slaking incubator. The gas phase in the flasks was either 95% O2-5% CO2 (oxygenated) or 95% N2-5% CO2 (hypoxia). In experiments in which muscles were incubated with 0.1 μM epinephrine, ascorbate (0.5 mg/ml) was added to the KHB. After incubation, muscles were blotted on damp filter paper, clamp frozen with aluminum tongs cooled in liquid N2, and stored at -70°C until analyzed.

**Analytical methods.** Frozen muscle samples were weighed and homogenized in 3 M HClO4. An aliquot of the HClO4 homogenate was used for measurement of glycogen with the amyloglucosidase method (32). The remaining homogenate was centrifuged at 3,000 g for 10 min at 4°C. The supernatant was neutralized with 2 N KOH containing 0.4 M imidazole and 0.4 M KCl and centrifuged at 4°C in a Microfuge. The neutralized supernatant was used for measurement of ATP (27), phosphocreatine (26), glucose 6-phosphate (28), and P1 (40) by enzymatic methods. AMP and IMP were measured by high-performance liquid chromatography (41), separation was made on a reverse-phase column (Nucleosil C18), and the mobile phase was 100 mM potassium phosphate buffer (pH 6.2).

For determination of glycogen phosphorylase activity, frozen muscle samples, weighing ~20 mg, were homogenized at -10°C in 0.2 ml of 100 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.5) containing 60% glycerol, 50 mM potassium fluoride, and 10 mM EDTA. Homogenates were then diluted with 0.8 ml of the same buffer without glycerol and homogenized further at 0°C. Phosphorylase activity was measured in the direction of glycogen breakdown at 30°C as described previously (44). Phosphorylase activity measured in the presence of 3 mM AMP is termed total activity, whereas activity measured in the absence of added AMP is defined as phosphorylase a. The total activity of phosphorylase was 37.4 ± 1.2 μmol·min⁻¹·g muscle⁻¹ in control muscles and was unaltered by either hypoxia or epinephrine. For studies of the inhibitory effect of 2-deoxyglucose 6-phosphate on phosphorylase activity in whole muscle homogenates, the assays were carried out in the presence of 40 and 100 μM AMP and in the absence of added AMP.

**Measurement of intracellular 2-deoxy-D-glucose accumulation.** Muscles were incubated at 35°C in 2 ml KHB containing 40 mM 2-deoxy-D-[1,2-3H]glucose (150 μCi/μmol, ICN Radiochemicals), 2 mM sodium pyruvate, and 500 μU/ml insulin for 120 min. The muscles were then washed for two 10-min periods in KHB containing 40 mM mannitol to remove free 2-deoxy-D-glucose (2-DG) from the extracellular space. Frozen muscles were weighed and homogenized in 1 ml 5% trichloroacetic acid (TCA). A portion of the homogenate was used for measurement of glycogen (32), and the remainder was centrifuged at 1,000 g. Aliquots of the supernatant were extracted with ether to remove TCA. The samples were vortexed for 10 s with an equal volume of diethyl ether and a total of four times; the residuum ether was then removed with a stream of N2 gas. Free and phosphorylated 2-DG were separated using 0.5 × 4 cm Dowex-1 columns (chloride form, 200-400 mesh; Sigma Chemical) prepared in Pasteur pipettes and equilibrated with H2O as described previously (24). Briefly, a 300-μl sample was loaded onto the column; the column was then washed with 2 ml of H2O to remove free 2-DG. Next, phosphorylated 2-DG was eluted from the column with 3 ml of 1.0 N HCl. The HCl eluates were neutralized with 10 N NaOH, and all of the samples were adjusted to the same volume. Radioactivity was determined by scintillation counting.

**Statistics.** The significance of differences between two groups was evaluated using Student’s paired t test. For multiple comparisons, a one-way analysis of variance (ANOVA) was employed. When ANOVA revealed significant differences, post hoc analysis was performed using Newman-Keuls multiple range test.

**RESULTS**

**Glycogenolysis and percentage of phosphorylase a.** Incubating epitrochlearis muscles in oxygenated KHB for 80 min had no significant effect on the concentrations of glycogen (27.3 ± 1.7 before vs. 28.6 ± 2.4 μmol/g after), phosphocreatine (16.0 ± 1.1 vs. 16.0 ± 1.0 μmol/g) or P1 (3.9 ± 0.5 vs. 4.9 ± 0.7 μmol/g). As shown in Fig. 1, hypoxia resulted in a progressive ~70% decrease in epitrochlearis muscle glycogen concentration over an 80-min period. An initial more rapid decrease over the first 10 min was followed by a slower steady decline. The large decrease in muscle glycogen concentration caused by hypoxia occurred in the absence of phosphorylase conversion to the α form (Table 1). There was a small but significant decrease in percentage of phosphorylase a during 40 min of hypoxia, from 10.9 ± 1.0 to 8.1 ± 0.3% (P < 0.02).

**Effects of hypoxia on muscle P1, glucose 6-phosphate, AMP, and IMP concentrations.** To obtain insights regarding the mechanisms responsible for rapid glycogenolysis in hypoxic muscle despite no increase in percentage of phosphorylase a, we determined the time course of the changes in the concentrations of P1, glucose 6-phosphate, AMP, and IMP. As shown in Fig. 1, P1 concentration increased rapidly and roughly mirrored the decline in phosphocreatine concentration during the first 40 min of hypoxia. It is of interest that the maximal rate of glycogenolysis was attained well before the maximal increase in P1 concentration. After 5 min of hypoxia, when the rate of glycogenolysis was at its peak, P1 had increased from 3.8 to 8.6 μmol/g muscle, whereas between 10 and 20 min, when P1 concentration averaged 15.7 μmol/g, glycogenolysis had already slowed (Fig. 1).

As shown in Table 2, muscle AMP concentration doubled in response to hypoxia, attaining a steady-state concentration of 0.23 μmol/g within 5 min. Despite the persistent increase in AMP concentration, the rate of glycogenolysis slowed after 10 min. There was also a small statistically insignificant increase in the concentration of phosphocreatine and IMP concentrations. The putative overshoot in muscle IMP concentration results from a progressive decrease in IMP, the major product of IMP synthase activity.

![Fig. 1](http://ajpendo.physiology.org/DownloadedFrom/10.22033/33338027/2017)
Table 1. Effects of hypoxia and epinephrine on phosphorylase a and glycogen concentration in epitrochlearis muscle

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time, min</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenated – glucose</td>
<td>%Phosphorylase a</td>
<td>10.4±1.1</td>
<td>10.9±1.0</td>
<td>10.4±0.8</td>
<td>9.7±0.3</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>30.0±1.4</td>
<td>29.7±1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxic</td>
<td>%Phosphorylase a</td>
<td>10.9±1.0</td>
<td>10.1±1.1</td>
<td>10.4±0.8</td>
<td>9.7±0.3</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>33.7±2.4</td>
<td>29.4±1.5</td>
<td>27.2±2.0</td>
<td>21.5±2.2</td>
</tr>
<tr>
<td>Oxygenated + epinephrine</td>
<td>%Phosphorylase a</td>
<td>10.1±0.7</td>
<td>65.1±5.0</td>
<td>64.0±3.5</td>
<td>66.2±4.9</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>30.9±1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxic + epinephrine</td>
<td>%Phosphorylase a</td>
<td>10.9±0.7</td>
<td>58.5±2.1</td>
<td>61.6±1.7</td>
<td>59.6±0.6</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>32.8±1.7</td>
<td>26.3±2.5</td>
<td>20.4±2.2</td>
<td>14.3±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 muscles. Glycogen values are expressed as pmol glucose/g muscle.

Table 2. Muscle ATP, AMP and IMP concentrations during 20 min of hypoxia

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>5.52±0.19</td>
<td>5.28±0.14</td>
<td>5.00±0.12</td>
<td>5.01±0.23</td>
</tr>
<tr>
<td>AMP</td>
<td>0.11±0.00</td>
<td>0.23±0.03*</td>
<td>0.20±0.03*</td>
<td>0.23±0.04*</td>
</tr>
<tr>
<td>IMP</td>
<td>0.11±0.01</td>
<td>0.16±0.02</td>
<td>0.16±0.01</td>
<td>0.17±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 muscles and are expressed as μmol/g muscle. * P < 0.05 vs. oxygenated.

Table 3. Effects of hypoxia and epinephrine on glucose 6-phosphate, phosphocreatine, and inorganic phosphate concentrations in epitrochlearis muscle

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time, min</th>
<th>0</th>
<th>5</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenated + epinephrine</td>
<td>Glucose-6-P</td>
<td>0.26±0.02</td>
<td>1.07±0.14*</td>
<td>1.35±0.09*</td>
</tr>
<tr>
<td></td>
<td>P_1</td>
<td>4.89±0.40</td>
<td>3.72±0.31</td>
<td>4.99±0.28</td>
</tr>
<tr>
<td></td>
<td>P_Cr</td>
<td>15.8±1.2</td>
<td>16.5±1.1</td>
<td>16.5±1.1</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>Glucose-6-P</td>
<td>0.26±0.03</td>
<td>0.20±0.02</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td></td>
<td>P_1</td>
<td>3.80±0.4</td>
<td>8.6±0.8*</td>
<td>15.7±1.8*</td>
</tr>
<tr>
<td></td>
<td>P_Cr</td>
<td>16.8±1.1</td>
<td>12.7±0.5*</td>
<td>3.9±0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 muscles and are expressed as μmol/g muscle. * P < 0.05 vs. time 0.

Inhibition of phosphorylase by 2-deoxyglucose 6-phosphate. It has been reported that 2-deoxyglucose 6-phosphate is an inhibitor of phosphorylase b but not phosphorylase a (21). We have confirmed this finding. As shown in Fig. 2, in the absence of added AMP 2-deoxyglucose 6-phosphate at concentrations of 10 and 20 mM had essentially no effect on phosphorylase activity. However, in the presence of 40 μM AMP, 10 mM 2-deoxyglucose 6-phosphate caused a decrease in phosphorylase activity to the level seen in the absence of AMP, i.e., a complete inhibition of phosphorylase b activity. In the presence of 400 μM AMP, 10 mM 2-deoxyglucose 6-phosphate caused a 50% decrease and 20 mM 2-deoxyglucose 6-phosphate caused a 68% decrease in phosphorylase activity.

It is still unclear whether noncovalent activation of phosphorylase b by AMP and/or IMP normally occurs in skeletal muscle. To evaluate the contribution of phosphorylase b activity to the glycogenolysis induced by hypoxia, we incubated paired epitrochlearis muscles with 40 mM 2-DG for 2 h in oxygenated medium so as to accumulate 2-deoxyglucose 6-phosphate intracellularly. One muscle of each pair was frozen while oxygenated to determine initial 2-deoxyglucose 6-phosphate concentration, and the other was frozen and analyzed after 60 min.
...of glycogenolysis. As shown in Table 4, the rate of glycogenolysis was slightly (~14%) slower in 2-DC-loaded muscles during 60 min of hypoxia. This difference was not statistically significant. The concentration of 2-deoxyglucose 6-phosphate in intracellular water of the 2-DC-loaded muscles was 19 mM initially and 11.5 mM after 60 min of hypoxia.

**DISCUSSION**

Our results show that rapid sustained glycogenolysis can occur in skeletal muscle without any increase in phosphorylase α activity. This finding, which is clearly incompatible with the classical model of the regulation of glycogenolysis (3, 6, 7, 20, 22, 23), is of interest because of the insights it provides regarding the regulation of glycogenolysis. Phosphorylase exists in two molecular forms. Phosphorylase b is inactive in the absence of AMP, and although AMP concentration in resting muscle appears to be above Michaelis constant (K_m) for its activation, phosphorylase b is thought to remain inactive, because most of the AMP is bound to protein (22, 30). Muscle contractions, by means of an increase in Ca^{2+}, and catecholamines, by means of increases in cAMP and in protein kinase A activity, activate phosphorylase kinase. This kinase in turn phosphorylates phosphorylase b, converting it to phosphorylase α, which is active in the absence of AMP (6, 7, 20, 23).

One of the premises on which the classical model of glycogenolysis was based is that essentially all of the phosphorylase in relaxed skeletal muscle is in the b form and that this explains why glycogenolysis does not occur in resting muscle (cf. Refs. 16, 20, 22, 23). However, despite improvements in the procedures for quick freezing and extraction of muscle, there has been no tendency for percentage of phosphorylase α levels measured in resting muscle to fall over the past 30 years (cf. Refs. 22, 36). Actually, the percentage of phosphorylase α in resting muscle may have been underestimated in some studies, and it now appears that a significant proportion of phosphorylase is in the α form in resting muscle under physiological conditions (cf. Ref. 22). If this is so, why does the phosphorylase α present in resting muscle not catalyze rapid glycogen breakdown?

Griffiths (22) and Chasiotis (4) have suggested that the explanation for this seeming paradox relates to availability of the substrate P_i. Although the concentration of P_i measured in perchloric acid extracts of muscle is usually 4–8 mmol/l of intracellular water, these high values appear to be due to breakdown of phosphocreatine during extraction (4). Nuclear magnetic resonance studies (14, 18) and aqueous alcohol extraction techniques (42) indicate that the true concentration of P_i in the cytosol of resting muscle is 2 mM. Furthermore, the P_i and phosphorylase α may be in different compartments in resting muscle. The K_m of muscle phosphorylase for P_i appears to be 26 mM (4). In this context, the hypothesis that limited availability of P_i serves as a brake and prevents net glycogenolysis in resting muscle despite significant phosphorylase activity seems reasonable.

Strong support for this concept is provided by the present finding that hypoxia, which causes an increase in P_i concentration, induces rapid sustained glycogenolysis in skeletal muscle without an increase in percentage of phosphorylase α. Further support comes from the finding that massive activation of phosphorylase to the α form by treatment of resting muscles with epinephrine, which does not cause an increase in P_i, results in negligible glycogenolysis (Table 1; Refs. 5, 37). In contrast, when P_i concentration in muscle is raised by hypoxia (Table 1) or prolonged contractile activity (37, 43), activation of phosphorylase with epinephrine significantly accelerates glycogenolysis.

Activation of phosphorylase kinase, by increases in cytosolic Ca^{2+} and β-adrenergic stimulation, resulting in conversion of phosphorylase b to α, was thought to be the mechanism by which glycogenolysis is regulated in muscle during exercise (3, 7, 19, 20, 23). However, a number of studies have shown that this mechanism is operative only transiently and that phosphorylase activation reverses during the first few minutes of contractile activity, with a decrease in percentage of phosphorylase α to, or below, the level found in resting muscle (1, 4, 9, 37, 39). In some studies involving continuous tetanic stimulation, reversal of phosphorylase activation coincided with development of fatigue (15, 33), and the decline in both contractile force and percentage of phosphorylase α could have been due to release of less Ca^{2+} from the sarcoplasmic reticulum because of impairment of excitation-contraction coupling. However, phosphorylase activation also reverses after a few minutes during steady-state muscle contractile activity in the absence of fatigue (9, 37).

Whether net glycogenolysis continues after phosphorylase activation reverses appears to depend on exercise intensity, availability of other substrates, and muscle fiber type. However, strenuous prolonged exercise does generally result in progressive glycogen depletion (2, 8). It was unclear how continuing glycogen breakdown is mediated after activation of phosphorylase has reversed. This phenomenon can now be explained in the context of the present results, which show that continuous glycogenolysis can occur in the absence of an increase in percentage of phosphorylase α activity above that found in resting muscle if the substrate P_i is made available, as it is during exercise and hypoxia.
Both AMP and IMP can stimulate phosphorylase a (1, 29). Moreover, it has been shown that 50 times as high a concentration of IMP is required to obtain the same degree of phosphorylase a activation as with AMP (1). In the present study, AMP concentration doubled after 5 min of hypoxia but no significant increase in IMP concentration was noted. Thus there is a possibility that the increase in AMP concentration during hypoxia may have caused some stimulation of phosphorylase a.

It is still not clear whether allosteric activation of phosphorylase b by AMP and/or IMP plays a role in glycogenolysis under physiological conditions, such as exercise and hypoxia. Evidence that allosteric activation of phosphorylase h can mediate glycogenolysis in muscle comes from studies of mice of the I strain, which lack phosphorylase kinase and are therefore unable to form phosphorylase a (17). Even so, stimulation of their muscles to contract results in glycogenolysis (17). However, the muscles of phosphorylase kinase-deficient mice have a number of metabolic adaptations that favor activation of phosphorylase b, and the results obtained on them cannot be directly extrapolated to normal muscle (21).

Nevertheless, the finding that phosphorylase b-mediated glycogenolysis can occur in muscle raised the question of the relative roles of phosphorylase a and b activities in catalyzing glycogen breakdown in hypoxic muscle. Roughly 90% of the AMP in skeletal muscle appears to be protein bound (30). If this was the case, the concentration of free AMP in the cytosol of the hypoxic muscles in this study was 40 μM. Based on the measurements made in vitro (Fig. 2), the concentration of 2-deoxyglucose 6-phosphate present in the muscles loaded with 2-DG, i.e., 11.5-19 mM, should have completely inhibited phosphorylase b activity in the hypoxic muscles. The finding that the rate of glycogenolysis was decreased by only 14% (Table 4) in the 2-DG-loaded muscles provides evidence that glycogenolysis in hypoxic skeletal muscle is mediated predominantly by phosphorylase a activity, with phosphorylase b activity possibly making a small additional contribution.

The rate of glycogenolysis peaked before the maximal increase in P1 was attained in the hypoxic muscles (Fig. 1), and glycogenolysis slowed after 10-20 min despite maintenance of a constant elevation of P1. A slowing of glycogenolysis occurred even in the epinephrine-treated hypoxic muscles in which percentage of phosphorylase a was maintained at 60% of total activity. Thus, although our results support the concept that unavailability of P1 prevents net glycogenolysis in resting oxygented muscles and provide evidence that the increase in P1 mediates glycogenolysis in hypoxic muscle, some other factor or factors appear to limit glycolytic rate after 10-20 min of hypoxia. This finding is in keeping with evidence from other studies that glycogenolysis slows as muscle glycogen concentration decreases (38, 43). One possible explanation for this slowing could be that as glycogen is degraded, the glycogenolytic enzymes bound to the outer surface of the glycogen molecule are released and that this change in the physical relationship between phosphorylase and its substrate could result in less efficient catalysis of glycogenolysis. A second possibility that seems worth consideration is that as glycogenolysis proceeds and an increasing number of limit dextrins are formed, transferase and debranching enzyme activities could become limiting.

In conclusion, our results show that hypoxia induces rapid glycogenolysis in skeletal muscle in the absence of any increase in percentage of phosphorylase a, whereas a sixfold increase in percentage of phosphorylase a induced by epinephrine treatment causes minimal glycogen breakdown in oxygenated muscle. Hypoxia resulted in increases in muscle P1 concentration whereas epinephrine did not. Loading muscles with 2-deoxyglucose 6-phosphate to inhibit phosphorylase b activity had little effect on the rate of glycogenolysis in hypoxic muscles. These findings provide evidence that 1) there is sufficient phosphorylase in the a form in resting muscle to mediate rapid glycogenolysis, 2) unavailability of P1 prevents net glycogenolysis in resting, oxygenated muscle, and 3) increases in P1 and/or AMP can induce and maintain glycogenolysis in muscle in the absence of an increase in percentage of phosphorylase a.

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