Lactate and contractile force in frog muscle during development of fatigue and recovery

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FITTS, ROBERT H., AND JOHN O. HOLLOSZY. Lactate and contractile force in frog muscle during development of fatigue and recovery. Am. J. Physiol. 231(2): 430-433. 1976. -The relationship between lactic acid concentration and twitch tension was reevaluated in electrically stimulated frog sartorius muscle. In muscles stimulated under anaerobic conditions at a rate of 30 stimuli/min, contractile force decreased to 36% of the initial value in 15 min. Concomitantly lactate increased from 3.3 to 18.7 μmol/g of muscle. The correlation between the increase in lactate and the decrease in contractile force was significant (r = -0.99, P < 0.00001). Recovery occurred in two phases. A rapid increase in contractile force, which represented 20% of the total recovery, took place during the first 15 s and occurred concomitantly with an increase in ATP from 3.9 to 4.6 μmol/g. Lactate concentration did not change significantly during this period. The second phase of recovery of contractile force was complete in 50 min. Lactate concentration and contractile force were significantly correlated during recovery (r = -0.92, P < 0.00001). However, recovery of contractile force lagged behind the decrease in lactate; a given concentration of muscle lactate was associated with a higher contractile force early during development of fatigue than late during recovery.

glycogen; ATP; phosphocreatine

ALTHOUGH MUSCLE FATIGUE has been the subject of considerable investigation, there is still relatively little known regarding its causes. One factor that has been postulated to cause fatigue during muscular work is the accumulation of lactic acid (3, 8, 11, 12, 16). Much of the evidence for the hypothesis that high concentrations of lactic acid in muscle cause fatigue came from the studies of Hill and Kupalov (7, 8). The interpretation of Hill and Kupalov's studies is open to question, because they did not measure lactate but estimated its concentration in frog sartorius muscles from the decrease in twitch tension during electrical stimulation (7, 8). The relationship between intramuscular lactic acid concentration and muscle fatigue in frog sartorius muscle does not appear to have been systematically reevaluated now that simple, accurate methods for lactate analysis are available. For the purpose of this study we use the term fatigue to mean a decrease in the performance capacity of muscle. As an indicator of muscle fatigue we have used a decrease in twitch tension. We use the term twitch tension interchangeably with contractile force.

As a first step in evaluating the role of lactic acid accumulation in muscle fatigue, we undertook the present study to determine whether there is a close correlation between lactic acid concentration and contractile force in frog sartorius muscle. Measurements were made both during development of fatigue and during the recovery process. We have evaluated the results in the context of the concomitant responses of muscle ATP, phosphocreatine, and glycogen.

METHODS

Frog care and handling of tissues. Northern frogs (Rana pipiens p. pipiens) were obtained from Mogul-Ed, Oshkosh, Wis., and kept in a large aquarium at room temperature (22-25°C). They were fed a diet of crickets and mealworms daily for at least 2 wk to ensure adequate muscle glycogen stores. Frogs were pithed and sartorius muscles were dissected out with the attachment to the pubic bone intact and stored overnight at 4°C in oxygenated solution. The bathing solution used in these experiments was a modification of the Krebs-Henseleit solution (13) and had the following composition: 89 mM NaCl, 25 mM NaHCO₃, 1.5 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.3 mM CaCl₂, and 5 mM glucose.

The procedures used for the electrical stimulation of frog sartorius muscles and the measurement of isometric tension have been described in detail previously (10). The pubic bone of the sartorius muscle preparation was tied to the lower end of a vertical Lucite rod containing two platinum electrodes. The tibial tendon was tied to a jeweler's chain, which connected it to a Sanborn model FTA-100 isometric force transducer (Hewlett-Packard Co., Waltham, Mass.). Muscle length was adjusted so as to obtain 1 g of resting tension. The mounted muscle was immersed in 15 ml of the solution in a test tube suspended in a water bath maintained at 22°C. The muscles were stimulated under anaerobic conditions produced by gassing the solution with a 95% N₂ and 5% CO₂ mixture from which traces of O₂ were first removed by passing it through a solution of vanadyl sulfate. After a 30-min equilibration period, the muscles were stimulated directly with supramaximal square wave pulses of 3 ms duration at a rate of 30 stimuli/min with a Grass model 54K stimulator (Grass Instruments, Quincy, Mass.). Isometric twitch tension was recorded on a Sanborn recorder, model 301 (Hewlett-Packard Co.).
In the studies on the development of fatigue, after 2, 6, 10, or 15 min of stimulation the solution in which the muscle was immersed was removed and the contracting muscle was quick-frozen in isopentane cooled in liquid N\textsubscript{2}. Control muscles were also mounted on the electrode holder and immersed in solution gassed with 95% N\textsubscript{2} and 5% CO\textsubscript{2} for 30 min. They were then given three stimuli spaced 10 s apart to determine initial twitch tension (defined here as the average of the 3 twitches) and frozen 1 min later.

In the studies of recovery from fatigue, all the muscles were stimulated for 15 min at a rate of 30 stimuli/min under anaerobic conditions as described above. Just prior to cessation of stimulation, the solution gassed with 95% N\textsubscript{2} and 5% CO\textsubscript{2} was replaced with oxygenated solution. The muscles were permitted to recover in oxygenated solution for either 0.25, 0.50, 1.0, 2.0, 6.0, 10, 20, or 30 min or until isometric twitch tension had returned to the initial (prefatigue) level (complete recovery took 50 ± 3 min), before they were frozen in isopentane cooled in liquid N\textsubscript{2}. The extent of recovery of contractile force was evaluated by the administration of a single stimulus immediately prior to the time the muscle was frozen.

**Assay methods.** The frozen muscle samples were weighed at -20°C. A 20- to 25-mg portion was stored at -90°C until used for determination of glycogen concentration. Muscle water content was determined by drying 10- to 20-mg portions of muscle to constant weight at 90°C. The remaining muscle was pulverized and extracted with perchloric acid as described by Williamson and Corkey (20). The extracts were analyzed for lactate by the enzymatic method of Hohorst (9), for ATP as described by Lamprecht and Trautschold (14), and for phosphocreatine by the procedure of Ennor and Rosenberg (2).

**RESULTS**

There was no difference in water content among the muscles that were quickly frozen after either 0, 2, 6, 10, or 15 min of stimulation. Water made up 82.5% of the total weight of the quickly frozen muscles; freshly isolated muscles and muscles bathed in the solution and blotted before freezing had a water content of 80%. This difference was assumed to be due to the solution covering the quickly frozen (unblotted) muscles.

The concentrations of lactate, ATP, phosphocreatine (PC), and glycogen are given per gram wet weight of muscle, corrected for the excess solution, as this seems the most physiologically relevant way of expressing the results. The concentration of lactate in the solution surrounding the muscles never exceeded 0.1 μmol/ml.

**Changes in contractile force, lactate, ATP, and PC concentrations during development of fatigue in isolated frog sartorius muscles.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Contractile Force, % of Control</th>
<th>Lactic Acid, μmol/g</th>
<th>Phosphocreatine, μmol/g</th>
<th>ATP, μmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (16)</td>
<td>100</td>
<td>3.32 ± 0.51</td>
<td>15.64 ± 0.37</td>
<td>5.15 ± 0.14</td>
</tr>
<tr>
<td>Fatigue development (under anaerobic conditions)</td>
<td></td>
<td></td>
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<tr>
<td>2 min Stimulation (5)</td>
<td>100 ± 1</td>
<td>4.70 ± 0.46</td>
<td>6.01 ± 0.56</td>
<td>4.48 ± 0.21</td>
</tr>
<tr>
<td>6 min Stimulation (7)</td>
<td>77 ± 1</td>
<td>10.44 ± 0.49</td>
<td>5.98 ± 0.88</td>
<td>4.87 ± 0.28</td>
</tr>
<tr>
<td>10 min Stimulation (9)</td>
<td>59 ± 1</td>
<td>14.85 ± 0.58</td>
<td>7.51 ± 0.32</td>
<td>4.25 ± 0.19</td>
</tr>
<tr>
<td>15 min Stimulation (10)</td>
<td>36 ± 1</td>
<td>18.66 ± 0.86</td>
<td>2.60 ± 0.43</td>
<td>3.32 ± 0.17</td>
</tr>
<tr>
<td>Recovery (in oxygenated Ringer solution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.25 min Recovery (8)</td>
<td>49 ± 1</td>
<td>10.69 ± 1.42</td>
<td>3.96 ± 0.30</td>
<td>6.61 ± 0.27</td>
</tr>
<tr>
<td>0.50 min Recovery (9)</td>
<td>53 ± 1</td>
<td>17.23 ± 1.20</td>
<td>4.08 ± 0.53</td>
<td>6.92 ± 0.14</td>
</tr>
<tr>
<td>1 min Recovery (9)</td>
<td>56 ± 1</td>
<td>16.40 ± 1.08</td>
<td>4.30 ± 0.53</td>
<td>4.46 ± 0.13</td>
</tr>
<tr>
<td>2 min Recovery (10)</td>
<td>56 ± 1</td>
<td>15.76 ± 1.03</td>
<td>7.76 ± 0.95</td>
<td>4.55 ± 0.16</td>
</tr>
<tr>
<td>6 min Recovery (7)</td>
<td>64 ± 2</td>
<td>16.26 ± 1.18</td>
<td>11.09 ± 0.08</td>
<td>4.78 ± 0.19</td>
</tr>
<tr>
<td>10 min Recovery (6)</td>
<td>56 ± 2</td>
<td>14.17 ± 1.22</td>
<td>11.86 ± 0.71</td>
<td>4.62 ± 0.29</td>
</tr>
<tr>
<td>20 min Recovery (8)</td>
<td>65 ± 2</td>
<td>7.75 ± 0.74</td>
<td>13.13 ± 0.46</td>
<td>5.12 ± 0.25</td>
</tr>
<tr>
<td>30 min Recovery (13)</td>
<td>77 ± 2</td>
<td>4.74 ± 0.50</td>
<td>15.88 ± 0.74</td>
<td>5.18 ± 0.15</td>
</tr>
<tr>
<td>Complete Recovery*</td>
<td>100 ± 3</td>
<td>3.49 ± 0.59</td>
<td>15.50 ± 0.59</td>
<td>5.09 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of muscles per group is given in parentheses. * 50 ± 3 min.

**FIG. 1.** Changes in contractile force and in lactic acid, ATP, and PC concentrations during development of fatigue in isolated frog sartorius muscles at 30 shocks/min for 15 min under anaerobic conditions and during subsequent recovery in oxygenated solution.

In tension was statistically significant ($r = -0.99, P < 0.000001$).

Muscle PC concentration decreased approximately 85% during the first 10 min of stimulation and then leveled off. As can be seen in Fig. 1, the decrease in PC concentration followed a different time course than did the decrease in muscle contractile force; as a result, there was no statistically significant correlation between these two variables.

The concentration of ATP in the stimulated muscles also declined significantly (Fig. 1), with the greatest decrease in ATP occurring during the first 2 min. There was a further decrease in ATP between 6 and 15 min of
stimulation, and, although this decrease in concentration was small, there was a statistically significant correlation between ATP concentration and contractile force ($r = 0.82, P < 0.05$).

Recovery of contractile force to initial levels was a prolonged process (Table 1, Fig. 1), even though the fatigued muscles, which had been stimulated under anaerobic conditions, were transferred to oxygenated solution. Recovery occurred in two phases. A rapid increase in contractile force, which represented approximately 27% of the total recovery, took place during the first 30 s. No further change occurred for about 10 min, after which contractile force increased linearly, returning to the initial, prefatigue level in 50 ± 3 min.

No significant decrease in lactate concentration occurred during the initial 30 s of recovery; the rapid increase in contractile force during this period therefore cannot be attributed to a reduction in lactate level. By the end of the first 10 min of recovery, the concentration of lactic acid had decreased by approximately 4 μmol/g of muscle without any further change in contractile force (Fig. 1). Thereafter, a progressive decrease in lactate concentration coincided with the second, prolonged phase of recovery (Table 1, Fig. 1). There was a statistically significant, inverse correlation between lactic acid concentration and contractile force during the recovery period ($r = -0.92, P < 0.00001$).

Approximately 75% of the return of PC concentration toward the normal resting level occurred during the first 6 min of recovery; the major portion of this increase in PC took place between 1 and 6 min, a period during which contractile force did not change. The remainder of the increase in PC occurred between the 10th and 30th min of the recovery period (Table 1, Fig. 1).

A rapid increase in ATP concentration, which represented about 57% of the return to the resting level, occurred during the first 15 s of recovery and coincided with the first phase of the increase in contractile force (Table 1, Fig. 1). No further significant change in ATP level occurred until the period between 10 and 20 min of recovery during which ATP concentration returned to the control level. This second increase in ATP concentration occurred concomitantly with a large decrease in lactate concentration (Table 1, Fig. 1).

Muscle glycogen concentration remained high throughout the development of, and the recovery from, fatigue, averaging 10.1 ± 1.1 mg/g of muscle in control muscles, 7.2 ± 0.83 mg/g in muscles that had been stimulated for 15 min, and 6.7 ± 0.7 mg/g in muscles that had recovered for 50 min after 15 min of stimulation.

**DISCUSSION**

Although the mechanisms involved in the development of muscle fatigue are still poorly understood, it does seem clear that fatigue can have a variety of etiologies. Among the factors that seem important in determining the cause of fatigue are the intensity and the duration of muscular work. There is strong evidence that during prolonged, moderately heavy work, depletion of muscle glycogen stores can result in the development of muscle fatigue (1, 6). The present study was designed so that muscle glycogen levels remained high, and depletion of glycogen stores could be eliminated as a cause of fatigue.

Because ATP is the direct source of energy for muscle contraction, depletion of ATP will result in cessation of muscle contraction. Phosphocreatine plays a secondary role, functioning to maintain the concentration of ATP at a high level in the face of ATP hydrolysis (15). In the present study, the decrease in muscle ATP concentration was not very great. The lowest concentration of ATP was approximately 3.9 μmol/g after 15 min of stimulation. However, it is possible that during contractile activity the concentration of ATP in the region of the myofibrils might decrease more markedly than in the muscle as a whole, so that limited availability of ATP to myofibrillar ATPase could impair contractile function despite only a moderate decrease in total muscle ATP content.

There was a close relationship between an increase in contractile force during the first 15 s of recovery and an increase in ATP concentration (from 3.9 to 4.6 μmol/g). This finding is compatible with the interpretation that the small decrease in ATP concentration during the last 5 min of stimulation contributed to the development of fatigue and that this effect was reversed by the increase in ATP during the first 15 s of recovery. A second possibility is, of course, that the correlation between the increases in ATP concentration and contractile force may be coincidental and not due to a cause and effect relationship. At all the other times when contractile force was below control levels during development of fatigue and during recovery, the concentration of ATP was not significantly different from, or higher than, the value obtained after 2 min of stimulation (approximately 4.5 μmol/g). This concentration of ATP was sufficient to permit development of a contractile force slightly above the control value after 2 min of stimulation (Table 1).

The concept that accumulation of lactic acid causes muscle fatigue has considerable appeal, because there is evidence for at least two mechanisms by which a decrease in intracellular pH could interfere with contractile function. Fuchs et al. (4) have found that an increase in H+ concentration interferes with Ca2+ binding to troponin by lowering the apparent binding constant. In another study, Nakamura and Schwartz (17) found that a decrease in pH increases the Ca2+-binding capacity of the sarcoplasmic reticulum. Both mechanisms would function to decrease the number of calcium ions bound to troponin during excitation-contraction coupling. This would reduce the number of active interactions between actin and myosin and thus could decrease contractile force. In addition, phosphofructokinase activity is inhibited by a decrease in pH (19), and accumulation of lactic acid could, by this means, slow glycolysis during intense muscular work. This mechanism could explain the finding of a further decrease in muscle ATP concentration during the last 5 min of stimulation, despite a decrease in contractile force and therefore a decrease in the rate of ATP hydrolysis.

Hill’s hypothesis that lactic acid accumulation causes
muscle fatigue is based on rather limited data and has been seriously questioned (cf. 18). The results we obtained during the development of fatigue show a remarkably high negative correlation between muscle lactate concentration and contractile force \((r = 0.99, P < 0.000001)\). This finding, by itself, supports Hill’s hypothesis that lactic acid accumulation results in development of muscle fatigue. The correlation between lactate concentration and muscle fatigue during recovery, while not as high as during development of fatigue, is still statistically significant \((r = -0.92, P < 0.00001)\). Although it seems foolhardy to suggest that correlations statistically significant at such a high level are coincidental, a careful evaluation of the recovery data indicates that the relationship between lactate concentration and contractile force is by no means as clear cut as the results of the statistical analysis would suggest.

As discussed earlier, the rapid phase of recovery of contractile force during the first 15 s after cessation of stimulation cannot be explained on the basis of a decrease in lactate concentration. Therefore, some factor other than lactate accumulation must have contributed to the development of fatigue; this is not evident from the data obtained during the period of stimulation. Furthermore, the return of contractile force to the control level lags markedly behind the decrease in muscle lactate (Table 1). For example, after 30 min of recovery contractile force was still 23\% below the control level despite a lactate concentration of only 4.7 \(\mu\text{mol/g}\) at the same concentration of lactate after 2 min of stimulation, contractile force was 103\% of control. Thus, muscles having the same concentration of lactate develop different tensions during a twitch early during development of fatigue compared to late during recovery.

Although this discrepancy does not invalidate the hypothesis that lactate accumulation interferes with muscle contraction, it does indicate that the relationship is not a simple one in which a given concentration of lactate is associated with a fixed reduction in contractile force. It also makes it necessary to postulate that if the development of fatigue was largely due to accumulation of lactate, then some secondary effect induced by increased lactate must persist while lactate concentration is decreasing. Any future hypothesis regarding the effect of lactate on development of muscle fatigue therefore will have to include a mechanism to explain the finding that recovery of contractile force lags behind the decrease in lactate concentration.

We thank Mrs. May Chen for skillful technical assistance and Ms. Sandy Zigler for secretarial assistance.

This research was supported by Research Grant HD 01813 from the National Institute of Child Health and Human Development and by a grant from the Muscular Dystrophy Associations of America, Inc.

R. H. Fitts was supported by National Institutes of Health Postdoctoral Research Fellowship AM 00126.

Received for publication 10 November 1975.

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