Influence of physiological L(+)-lactate concentrations on contractility of skinned striated muscle fibers of rabbit

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Andrews, Mark Anthony W., Robert E. Godt, and Thomas M. Nosek. Influence of physiological L(+)-lactate concentrations on contractility of skinned striated muscle fibers of rabbit. *J. Appl. Physiol.* 80(6): 2060–2065, 1996.— These experiments investigated the effects of physiological concentrations of L(+)-lactate on the contractility of chemically skinned rabbit fast-twitch psoas, slow-twitch soleus, and cardiac muscles at pH 7. L(+)-Lactate depressed maximal calcium-activated force (F_{max}) of all muscles studied within the range of 5–20 (slow-twitch muscle) or 5–25 mM (fast-twitch and cardiac muscles). F_{max} of fast-twitch fibers was inhibited to the greatest degree (9% in K_2 creatine phosphate solutions). In all of these muscle types, F_{max} returned to control levels as L(+)-lactate was increased to 30–50 mM. Substitution of neither D-lactate nor propionate for L(+)-lactate significantly altered F_{max}. In addition, with the exception of fast-twitch muscle (where the Hill coefficient decreased), L(+)-lactate concentrations, which maximally inhibited F_{max}, did not affect the force vs. pCa relationship of muscles tested. These results demonstrate that L(+)-lactate significantly contributes to the depression of muscle function noted during lactic acidosis, directly inhibiting F_{max} of the contractile apparatus. This contribution is maximal in fast-twitch muscle where L(+)-lactate is responsible for as much as one-third of the depressant effect on F_{max} of the contractile apparatus noted during lactic acidosis.

skeletal muscle; cardiac muscle; contraction; calcium sensitivity

DURING PERIODS of increased muscular energy demand, e.g., with strenuous physical activity, there is a high rate of ATP utilization within intact muscle. The aerobic pathways of ATP production in fast-twitch skeletal muscle fibers may be unable to keep pace with energy demands because of limited oxidative capacity. This can be exacerbated by the hypoxic conditions that soon prevail because of limited ability of the cardiovascular system to meet oxygen demands. This situation is also enhanced by any pathological condition that may limit oxygen delivery to, or utilization by, the muscles. Intracellular stores of high-energy compounds, such as phosphocreatine (PCr), can buffer ATP levels for only a few seconds. Therefore, ATP production becomes highly dependent on anaerobic glycolysis, and L(+)-lactate concentrations within fast-twitch skeletal muscle fibers may increase to 30 mM or more (5, 31). L(+)-Lactate concentrations within the intracellular milieu of these fibers rise above 10 mM during even moderate, predominantly aerobic, exercise (7, 22). Once generated, L(+)-lactate concentrations are maintained throughout the duration of the exercise bout, decreasing only as the intensity of activity is markedly reduced or as exercise is terminated (14).

Because of the enzymatic capacity of fast-twitch skeletal muscle cells to utilize the anaerobic glycolytic pathways, L(+)-lactate is readily produced and diffuses out into the circulation, rapidly increasing the blood lactate levels into the millimolar range (20), with Osnes and Hermansen (30) finding blood lactate concentrations to be as high as 32 mM after exhaustive exercise. Under normoxic conditions, slow-twitch skeletal muscles and the myocardium take up L(+)-lactate from the blood when it is $\geq$0.2 mM (15). Furthermore, L(+)-lactate does not build up within these muscles to as great a degree as in fast-twitch glycolytic muscle fibers, serving as a preferential substrate for oxidative metabolism (20). However, under hypoxic conditions, L(+)-lactate will increase within slow-twitch muscles and the myocardium as well (23, 24), reaching concentrations in slow-twitch muscles about one-half of those found in fast-twitch muscles (11, 32). Furthermore, independent of the muscle fiber type, once L(+)-lactate production is initiated at a given workload, the intracellular concentration continues to increase as the exercise is maintained (13, 14), with a decline in L(+)-lactate levels occurring only when the exercise is reduced or terminated.

The decline in force generation noted during in vivo fatigue is due to summation of a number of changes within the muscle cell, at the sarcolemma and neuromuscular junction, and outside the muscle cell (nervous and vascular supply). Allen and co-workers (1) have reviewed fatigue and propose that during the initial onset of fatigue the maximal calcium-activated force (F_{max}) of the contractile apparatus (cross bridges) is directly affected and is responsible for a portion of the loss of force generation. The remaining effects on force generation are due to alterations of the calcium sensitivity of the contractile apparatus and of the calcium release and reuptake by the sarcoplasmic reticulum. The goal of the present research is to better understand the changes in the intracellular milieu that are responsible for the decrease in force generation and calcium sensitivity during fatigue by studying the effects of L(+)-lactate on the contractile apparatus of chemically skinned muscle fibers.

The increased intracellular H^+ concentration that accompanies L(+)-lactate production and other metabolites produced under fatigue conditions, e.g., P_i and ADP (10, 21), have been shown to adversely affect the contractility of skinned muscle fibers (9, 18). Recent evidence suggests L(+)-lactate has roles in muscular fatigue independent of pH (19); however, the direct

effects of physiological concentrations of L(+)-lactate on the contractile apparatus, independent of pH changes, have not been fully investigated. In the only previous investigation of L(+)-lactate on myofilament contractility (9), a high physiological level of 50 mM (the only concentration studied) was shown to increase F_{max} by a few percent. It was recently stated in a thorough review of cellular fatigue mechanisms by Fitts (12) that, "It is now generally recognized that the high inverse correlation between lactate and force is, for the most part, dependent on the high correlation between the lactate and the free H^+, and that the force-depressing agent is H^+ and not lactate." The purpose of the experiments reported herein was to investigate the direct effects of physiological concentrations of L(+)-lactate, at a constant pH of 7 and a constant (physiological) ionic strength of 200 mM, on the contractile properties of single chemically skinned slow- (soleus) and fast-twitch (psoas) skeletal muscles and thin strips of cardiac papillary muscle fibers of the rabbit.

METHODS

Rabbit single fast-twitch psoas fibers, slow-twitch soleus fibers, and bundles of muscle fibers from papillary muscles were used in these experiments. The rabbit was chosen as the experimental model because L(+)-lactate concentrations measured under physiological conditions in rabbit muscle have been shown to be similar to those encountered in humans (27). The rabbits were killed by pentobarbital sodium overdose, and small bundles of muscle fibers were excised and chemically skinned in a solution containing (in mM) 1 Mg^{2+} (free Mg^{2+} concentrations achieved by appropriate addition of MgCl), 2 Mg cytidine triphosphate (achieved by appropriate addition of MgCl and the acid form of cytidine triphosphate), 5 ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 20 imidazole, and 0.1 leupeptin and 0.5% (vol/vol) purified Triton X-100 nonionic detergent (Boehringer Chemical) by using potassium methanesulfonate ($MeSO_3^-$) [$KMeSO_3$; prepared from $HMeSO_3$ (Fluka Chemie) and 1 N KOH (Fisher Scientific)] to adjust ionic strength to 150 mM, with pCa > 8.5 and pH of 7. This solution was then mixed in a volume-to-volume ratio with glycerol, and the tissue was stored in this mixture at −20°C until used (within 2–3 wk).

In the experiments on skeletal muscles, a fiber bundle was removed from the freezer immediately before experimentation and bathed at room temperature (~22°C) in a solution containing (in mM) 1 Mg^{2+} (free Mg^{2+} concentrations achieved by appropriate addition of MgCl), 2 Mg-ATP (achieved by appropriate addition of MgCl and the acid form of ATP), 5 EGTA, and 20 imidazole and 0.5% vol/vol purified Triton X-100 nonionic detergent. With appropriate $KMeSO_3$ added, this solution had a total ionic strength of 150 mM, with pCa > 8.5 and pH of 7. Single fibers were dissected from the fiber bundle and attached between the arm of an optoelectronic force transducer and a stationary arm by wrapping the fiber around sand-blasted hooks. The fiber was stretched to a sarcomere length of 2.6 μm for all skeletal muscle fibers, as determined by He-Ne laser diffraction. At this sarcomere length, the preparations generate near-maximal force and the calcium affinity to the troponin binding sites (and thus calcium sensitivity of the contractile apparatus) is maximal and no different than at longer sarcomere lengths (28). For further experimental details see Andrews et al. (3) and Godt and Nosek (18).

In the experiments with papillary muscles, small bundles (<200 μm in diameter) were dissected and attached by small microforceps to the force transducer (Scientific Instruments, Heidelberg, Germany) and a stationary post. Because a diffraction pattern cannot be detected from such fiber preparations (26), they were initially stretched until a resting force was perceived and were held at this length for the course of the experiment as described previously (18). Otherwise, the cardiac fibers were treated identically to the skeletal muscle fibers.

The experimental solutions utilized were formulated according to microcomputer programs written in Turbo Pascal (Borland International). These programs solve the set of simultaneous equations describing the multiple equilibria of ions in the solutions by using appropriate association constants (4, 16, 18). For L(+)-lactate, binding constants for Mg^{2+} (8.51 M^{-1}), Ca^{2+} (11.75 M^{-1}), and H^+ (4.57 × 103 M^{-1}) were taken from Martell and Smith (25). All L(+)- and D-lactate and propionate concentrations given are for the free (unbound) anion form.

After being mounted on the force transducer, fibers were transferred through a series of experimental solutions contained in rows of Plexiglas troughs (2.5 ml). Triton X-100 (0.5% vol/vol) was added to each trough to reduce surface tension of the solutions and ensure full permeabilization of the membranes. The low-calcium (pCa > 8.5) control solution contained (in mM) 1 Mg^{2+} (achieved by appropriate addition of MgCl), 1 Mg-ATP (achieved by appropriate addition of MgCl and the acid form of ATP), 15 Na_2PCr, 5 EGTA, 20 imidazole, and ~100 U/ml creatine kinase, at pH 7.00 (± 0.01 unit), to which $KMeSO_3$, K_2PCr (Sigma Chemical), or tetramethylammonium (TMA) $MeSO_3$ [TMA $MeSO_3$; prepared from $HMeSO_3$ (Fluka Chemie) and TMA OH (Sigma Chemical)], was added to maintain a near-physiological constant ionic strength of 200 mM (17). It has been previously determined (3) that force generation of skinned fibers is extremely sensitive to ionic strength and that a constant ionic strength must be maintained among bathing solutions used for a set of experiments. At an ionic strength of 200 mM, the solutions contained enough $KMeSO_3$ (or K_2PCr or TMA $MeSO_3$) so that as new solutions were prepared to include the L(+)-lactate salt, an equal amount of $KMeSO_3$ (or K_2PCr or TMA $MeSO_3$) could be left out of the solution, up to and including the 50 mM concentration needed to accomplish these experiments. The initial experiments were conducted three times by using three different salts to adjust ionic strength to ensure that no specific ionic effects, caused by the presence of any one ion or its interaction with L(+)-lactate, gave us erroneous results or misled our conclusions. $KMeSO_3$ was used because it was previously found that fibers bathed in solutions containing $KMeSO_3$ functioned like fibers bathed in more physiological (6) yet more expensive and more labile K_2PCr-containing solutions (3). By choosing $KMeSO_3$ as our standard, the use of K_2PCr allowed us to vary the anion, maintaining the same cation, to assess any specific anion effect of $MeSO_3^-$, including any specific interactions with L(+)-lactate. In a similar manner, the use of TMA $MeSO_3$ allowed us to vary the cation, maintaining the same anion and allowing us to assess any specific effect of K or interactions with lactate. In addition, $KMeSO_3$, K_2PCr, and TMA $MeSO_3$ were chosen because they minimize swelling of the myofilament lattice of skinned fibers (3). The use of a 200 mM total ionic strength is consistent with previous experimental protocols by this group (3, 18). The activation solutions were similar but contained appropriate amounts of $CaCl_2$ to adjust the concentration of free calcium to the appropriate pCa values.

A fresh 0.5 M stock solution of L(+)-lactate or D-lactate or propionate (Sigma Chemical) was made (as the K or TMA salt, as appropriate to maintain a relatively constant cationic environment) immediately before the experimental solutions were mixed. Appropriate amounts were added to attain concentrations of 0–50 mM as the $MeSO_3^-$ salt or K_2PCr was proportionally reduced to maintain a constant ionic strength of 200 mM. All pH adjustments were made with the appropriate acid or base.

Before the effects of lactate and propionate on F_{max} were evaluated, the calcium concentration at which F_{max} was achieved under control conditions was determined for each fiber by successive activation in solutions of pCa 5, 4.5, and 4 (F_{max} was always achieved over this range of calcium concentrations, with the exact pCa of F_{max} showing variability among fibers). All consequent activations for a given fiber were carried out at the pCa that generated F_{max}.

As illustrated for a representative fiber in Fig. 1, fibers were activated by using a continuous (plateau) activation procedure that involved an initial control activation followed by activation through all lactate or propionate concentrations (0–50 mM) and then by a return to the control activation solution (to control for any alteration in F_{max}). All responses to lactate or propionate were normalized to the average of the F_{max} generated in solutions containing zero lactate or propionate (control) before and after passage of the preparation through the lactate- or propionate-containing solutions (there was usually very minimal, if any, difference between the initial and final control contractions). A few sampled fibers were also activated via a protocol that involved repeated activation-relaxation cycles. This confirmed that there was no difference in F_{max} between the plateau and repeated-contractions procedures (results not shown).

Effects on the force vs. pCa relationship of the contractile apparatus by 25 mM L(+)-lactate were also investigated in progressively activated single psoas fibers and cardiac papillary muscle bundles and by 20 mM L(+)-lactate in single soleus muscle fibers at 200 mM total ionic strength (adjusted with $KMeSO_3$). The two parameters of calcium activation, the calcium concentration ($[Ca^{2+}]$) at which half-maximal activation occurred [Ca_{50} (K)] and the Hill coefficient (N; a measure of the slope of the force vs. pCa relationship), were determined by least squares fit to a Hill equation of the form (16)

$$\%F_{max} = 100\,[Ca^{2+}]^N/(K^N + [Ca^{2+}]^N)$$

Throughout the experiments, each fiber acted as its own control. Means and SEs were calculated, and analysis of variance with Student-Newman-Keuls multiple comparisons and appropriate paired one- and two-tailed Student's t-tests were run (alpha values were usually 0.05 or 0.01 for specific tests) to determine the significant differences among results. All force data are reported as a percentage of the mean F_{max} at 200 mM ionic strength with no L(+)- or D-lactate or propionate present.

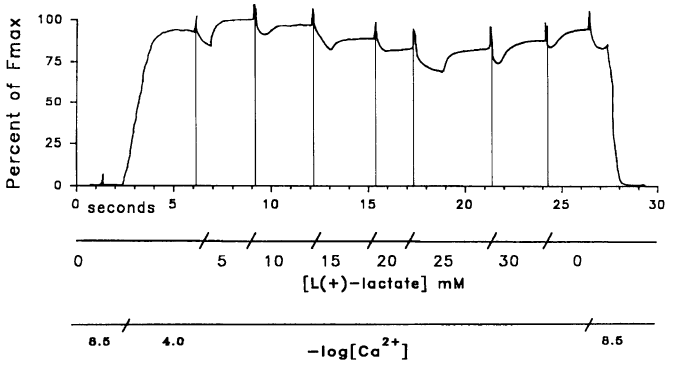

Fig. 1. Psoas muscle fiber activation record. Effect of increasing L(+)-lactate concentrations on force generation of rabbit single chemically skinned psoas muscle fibers in presence of K methanesulfonate ($KMeSO_3$). Brackets denote concentration. F_{max}, maximal calcium-activated force.

Table 1. *Effects of L(+)-lactate on maximal calcium-activated force production of skinned rabbit psoas muscle fibers*

L(+)-Lactate Concentration, mM	Ionic Strength Regulator		
	$KMeSO_3$ ($n = 18$)	K_2PCr ($n = 6$)	TMA $MeSO_3$ ($n = 6$)
5	102.73 ± 0.86	96.72 ± 0.98*	100.09 ± 0.21
10	99.15 ± 1.13	94.90 ± 1.49*	100.30 ± 0.70
15	94.45 ± 1.55*	92.30 ± 1.11*	97.78 ± 0.38*
20	94.23 ± 2.14*	94.49 ± 0.96*†	95.44 ± 0.68*
25	95.08 ± 2.19*	95.57 ± 1.33*	93.69 ± 0.59*
30	97.92 ± 1.49*†	95.85 ± 1.34*	101.58 ± 0.48†
50	99.42 ± 0.78	100.51 ± 0.54†	100.28 ± 0.94

Values are means ± SE given in percent; n, no. of muscle fibers. $KMeSO_3$ and TMA $MeSO_3$, K and tetramethylammonium methanesulfonate, respectively; K_2PCr, K_2 phosphocreatine. *Significantly less force was generated at this L(+)-lactate concentration than in similar solution control solution containing 0 mM L(+)-lactate, $P < 0.01$. †Significantly greater force was generated at this L(+)-lactate concentration than in next lower concentration, $P < 0.01$.

RESULTS

Fast-twitch psoas fibers. As shown in Table 1, and in the representative record in Fig. 1, we found that at constant ionic strength and pH, and independent of the salt used to adjust ionic strength, increasing the concentration of L(+)-lactate has a biphasic effect on F_{max} of fast-twitch psoas fibers: F_{max} decreases in a linear manner, with the minimum force occurring between 15 and 25 mM L(+)-lactate. The concentration of L(+)-lactate at which the maximal depression of F_{max} occurred was variable among fibers. The average maximum depression was to 93% of control in $KMeSO_3$, to 91% of control in K_2PCr, and to 93% of control F_{max} in TMA $MeSO_3$. F_{max} of psoas fibers in the presence of 50 mM L(+)-lactate always returned to a level that did not significantly differ from control, independent of the salt used to adjust ionic strength (99% in $KMeSO_3$, 100% in K_2PCr, and 100% of control F_{max} in TMA $MeSO_3$).

Figure 1 also illustrates that the effects of L(+)-lactate are completely reversible; F_{max} is altered as the muscle is exposed to different concentrations of L(+)-lactate and then returns to the control level when reexposed to control activation solution. There was no evidence of hysteresis under any condition. It was found that the depressant effects of L(+)-lactate are independent of *1*) the order in which fibers are exposed to the various concentrations of L(+)-lactate and *2*) whether a fiber is relaxed between activations in the different solutions or is continuously activated.

Propionate is a close structural analogue of L(+)-lactate (2-hydroxypropionate), lacking a hydroxyl group on the second carbon. Neither propionate nor D-lactate has a significant effect on F_{max} at concentrations up to

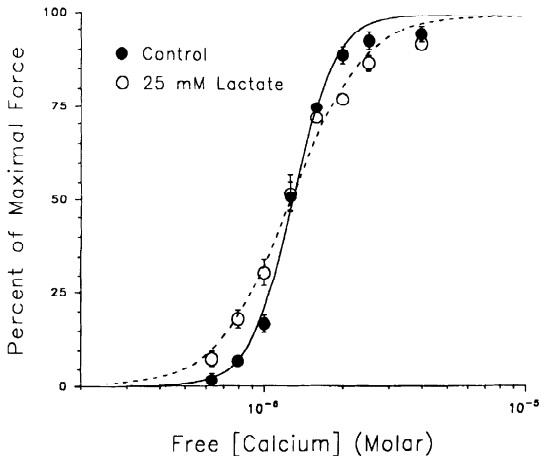

Fig. 2. Effect of L(+)-lactate on calcium sensitivity of rabbit single chemically skinned psoas muscle fibers at 200 mM ionic strength (adjusted with $KMeSO_3$). Values are means ± SE. Control, 0 mM L(+)-lactate.

30 mM. At 50 mM a small but significant decrease in F_{max} is observed in the presence of potassium propionate [perhaps due to a specific-ion effect of propionate (3)] but not with D-lactate.

As shown in Fig. 2, calcium sensitivity of the contractile apparatus of psoas fibers is altered in the presence of 25 mM L(+)-lactate (tested in $KMeSO_3$). When the force vs. pCa data are fit to the modified Hill equation, the N value for these fibers was significantly reduced by L(+)-lactate from 5.74 ± 0.39 to 4.17 ± 0.42 while there was no significant change in the Ca_{50} (≅1.3 μM under both conditions).

Slow-twitch soleus and cardiac papillary fibers. The effects of L(+)-lactate were also evaluated in slow-twitch soleus and cardiac papillary muscles by using $KMeSO_3$ to adjust ionic strength. Table 2 demonstrates that L(+)-lactate also has a significant biphasic inhibitory effect on the F_{max} in both of these fiber types. Similar to the effects on fast-twitch fibers, the concentration of L(+)-lactate at which the maximal depression of F_{max} occurred was variable among fibers. The average maximum depression was to 96% of control F_{max} in soleus and to 97% of control F_{max} in cardiac papillary fibers, with force returning to a level that did not significantly differ from control F_{max} when L(+)-lactate concentration was increased to 50 mM (100% for both soleus and cardiac papillary muscle). Figures 3*A* (soleus) and *B* (cardiac papillary) illustrate that L(+)-lactate (20 mM in soleus fibers and 25 mM in cardiac muscle) did not significantly affect the force vs. pCa relationship of either muscle type.

Table 2. *Effects of L(+)-lactate on maximal calcium-activated force production of rabbit single chemically skinned soleus fibers and cardiac papillary muscle fiber bundles*

L(+)-Lactate Concentration, mM	Soleus	Cardiac Papillary
5	100.70 ± 0.30	99.78 ± 0.24
10	99.55 ± 0.95	99.67 ± 0.37
15	97.47 ± 1.09*	99.23 ± 0.45
20	96.28 ± 1.12*	99.58 ± 0.22
25	98.78 ± 0.53*†	98.01 ± 0.51*
30	100.00 ± 0.43†	99.78 ± 0.24†
50	100.70 ± 0.69	100.81 ± 0.47

Values are means ± SE given in percent for 6 muscle fibers in each group. $KMeSO_3$ was used as ionic strength adjuster. *Significantly less force was generated at this L(+)-lactate concentration than in same solution with 0 mM L(+)-lactate, $P < 0.01$. †Significantly greater force was generated at this L(+)-lactate concentration than in next lower concentration, $P < 0.01$.

DISCUSSION

Many physiological functions are altered during the multifaceted process of fatigue. As a fatiguing stimulation is initiated in a muscle, F_{max} of the contractile apparatus (cross bridges) is depressed, significantly contributing to the loss of force generation by the muscle (1). The present results indicate that L(+)-lactate exerts a direct influence on the F_{max} of the contractile apparatus of chemically skinned striated muscle fibers, the maximal inhibitory effect occurring within the physiological range of 0–30 mM L(+)-lactate (27). As the concentration of L(+)-lactate was increased to 50 mM, F_{max} returned to its control value. L(+)-Lactate affected the F_{max} of fast-twitch psoas muscle

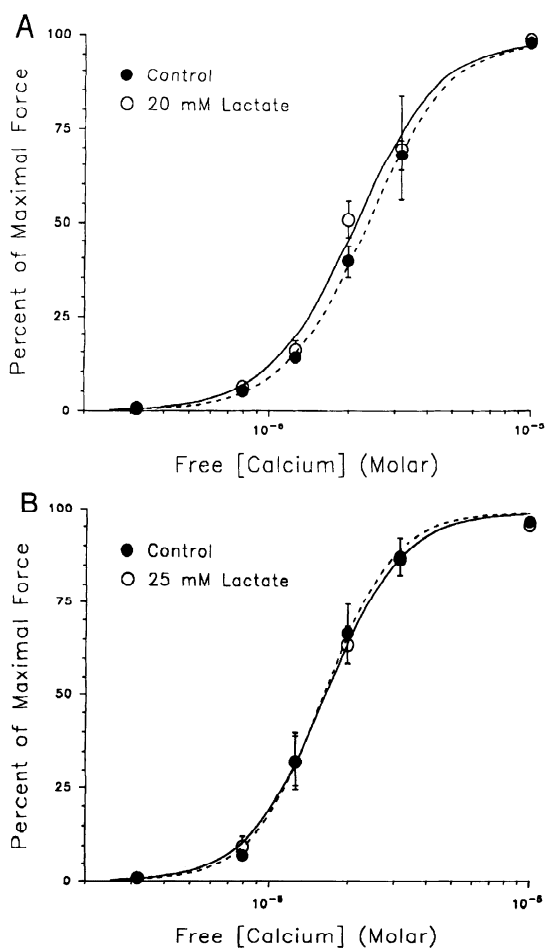

Fig. 3. *A*: effect of 20 mM concentration of L(+)-lactate on calcium sensitivity of rabbit single chemically skinned soleus muscle fibers. *B*: effect of 25 mM concentration of L(+)-lactate on calcium sensitivity of rabbit chemically skinned cardiac papillary muscle fiber bundles. All experiments were conducted by using solutions of 200 mM ionic strength (with $KMeSO_3$). Values are means ± SE.

fibers to the greatest extent, having a lesser effect on slow-twitch soleus and on cardiac papillary muscles. The similarity of the responses of slow-twitch and cardiac muscles to L(+)-lactate may be related to the fact that both contain slow-type myosin (33) compared with the fast-type myosin found in rabbit psoas. The concentration of L(+)-lactate that maximally affected F_{max} had no effect on the force-pCa relationship of either soleus or cardiac papillary muscle, and only slightly decreased the slope of the curve (N) in psoas muscle fibers.

The effect of L(+)-lactate on F_{max} of fast-twitch muscle fibers reported here differs from the only previous study of the effects of L(+)-lactate on skinned fibers by Chase and Kushmerick (9). They found that L(+)-lactate had a slight enhancing effect on the force-producing capacity of the contractile apparatus of fast-twitch single rabbit psoas muscle fibers. However, in that study, the effects of only a single high physiological concentration of 50 mM L(+)-lactate was evaluated, and the anion for which L(+)-lactate was substituted was acetate, an anion shown to be more deleterious to F_{max} of psoas muscle than either $MeSO_3^-$ or PCr (3), the substituted anions used in the present experiments. The removal of an anion that decreases F_{max} would be expected to cause an increase in F_{max}. This anion substitution effect would explain the results of Chase and Kushmerick. In our hands, the substitution of 50 mM L(+)-lactate for $MeSO_3^-$ or PCr^{2-} had no significant effect on F_{max}.

To ensure that the present findings are not dependent on the major salt added to the bathing solutions to maintain the ionic strength at 200 mM, a number of salts were used: *1*) $KMeSO_3$; *2*) K_2PCr, the major intracellular salt in muscles (6, 17); and *3*) TMA $MeSO_3$. These salts were chosen because they have minimal salt-specific effects on the contractile apparatus (3). Table 1 indicates that the biphasic influence of L(+)-lactate on F_{max} is independent of the anions substituted for L(+)-lactate.

The exact mechanism by which L(+)-lactate inhibits F_{max} is not known. However, the inhibition is highly specific to L(+)-lactate because two close structural analogues of L(+)-lactate, D-lactate and propionate, produced no significant inhibition of F_{max}. The inhibitory effect of L(+)-lactate is probably not due to an ion-specific protein-destabilization effect of L(+)-lactate on the contractile proteins. This conclusion is based on our previous study (3), which demonstrated that propionate had greater ion-specific inhibitory effects on F_{max} than did L(+)-lactate.

The return of F_{max} to near control levels at higher physiological concentrations of L(+)-lactate, independent of the salt used to maintain physiological ionic strength, was a consistent observation in all three muscle types studied here. The physical basis for this rebound effect is not obvious, although it is observed in other systems, including enzyme activities, that exhibit a biphasic response as salt or cryoprotectant concentrations are increased (8).

These results are in general agreement with in situ results reported by Hogan et al. (19). In their recent study, the infusion of L(+)-lactate into submaximally (30% of maximal oxygen uptake) contracting dog gastrocnemius to attain a blood lactate of 12–15 mM significantly reduced tension development independent of pH. However, due to the complexity of in situ preparations, they were unable to separate any direct effect of L(+)-lactate on the contractile apparatus from effects on one or more steps of the excitation-contraction coupling process or from indirect effects due to alteration of intracellular ionic strength. The skinned fiber preparation used in the present experiments not only enables us to maintain a constant pH but also allows us to maintain ionic strength ($\mu_s = \frac{1}{2}\Sigma c_i z_i^2$, where c_i is molar concentration of ion i and z_i is valence of ion i) at 200 mM. Such control enables us to separate the direct effect of L(+)-lactate from the effect of altered pH or ionic strength. The effects of L(+)-lactate reported by Hogan et al. are not likely to be due solely to an increase in ionic strength by L(+)-lactate. We have previously shown that when the ionic strength of solutions bathing the contractile apparatus of skinned muscle fibers is increased by 75 mM using the potassium salt of L(+)-lactate, F_{max} was only minimally inhibited. (3).

The concentrations of H^+ accumulated within muscles during fatiguing contractions (primarily due to lactic acid production and its consequent dissociation at physiological pH) have the capacity to decrease the F_{max} of skinned fast-twitch muscle by ~20% as pH is lowered from 7.0 to 6.6 (29). The maximal inhibition of F_{max} of skinned fast-twitch psoas fibers caused by L(+)-lactate is somewhat less, averaging ~8%. Therefore, the possible contribution of L(+)-lactate to the inhibitory effect of lactic acidosis [the combination of pH and L(+)-lactate effects] on F_{max} of fast-twitch muscle is estimated to be ~30% (8% ÷ 28%). Thus the L(+)-lactate produced during lactic acidosis does appear to have the capacity to decrease F_{max} of the contractile apparatus of fast-twitch muscle. Similarly, during lactic acidosis in slow-twitch and cardiac papillary muscle, L(+)-lactate may be responsible for ~23 and 10%, respectively, of the maximal inhibitory effect of lactic acidosis on F_{max} (29).

Therefore, in skinned muscle fiber preparations, L(+)-lactate itself appears to play a significant role in fatigue of all muscle fiber types. Furthermore, recent results by Westerblad and Allen (34), showing a clear dissociation between pH and alterations in F_{max} in vivo, suggest that L(+)-lactate may actually play a more prominent role in decreasing F_{max} during the process of lactic acidosis than was calculated here.

L(+)-Lactate was found to modulate the force vs. pCa relationship of the contractile apparatus of only fast-twitch skeletal muscle fibers, decreasing the slope (N) while leaving Ca_{50} unchanged (Fig. 2). As a result, calcium sensitivity of the fast-twitch fibers is increased at low calcium concentrations (micromolar and lower) and decreased at higher concentrations. An exact mechanism is difficult to propose at this time; however, it is possible that the increased sensitivity at low calcium concentrations may be one of the factors responsible for

the slowing of muscle relaxation noted during repetitive contractions of intact muscle fibers (34, 35).

In summary, at a constant pH and ionic strength, and within its physiological range of concentrations, we find that L(+)-lactate plays an active role in the fatigue process. It exerts a biphasic influence on F_{max} and decreases the slope of the force vs. pCa relationship of skinned fast-twitch skeletal muscle fibers. It also has a significant influence on F_{max} of skinned slow-twitch and cardiac muscle fibers while having no significant effect on their calcium sensitivity.

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