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Discrimination Between the Acidic and Molecular Effects of Lactate on Muscle Tension Development *

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Abstract: Fatigue can be defined as the decrease in the genesis of maximal muscle tension, and it may develop when metabolic end-products cannot be readily washed out. During anaerobic exercises, lactate accumulates in the muscle and blood, resulting in acidic pH in these tissues. Fatigue during lactate accumulation is generally attributed to its acidifying property; however, the inverse effect of lactate on muscle tension also at isopH values may be due to the molecular as well as the acidifying effects of this metabolite. In this study, we aimed to distinguish the molecular and acidifying effects of high lactate, both of which lead to decreased muscle tension development. Muscle tension developing in response to low frequency (0.5 Hz) supramaximal stimuli was recorded in the rat hemidiaphragm preparation. Rat Ringer solution (pH=7.33) was used as the control. Effects of high lactate concentration (high [La]) and acidic pH were evaluated by adding 20 mM of L-(+)-Lactate and 5 mM of acetic acid into the Ringer solution, respectively; pH values of the corresponding solutions were adjusted either by buffering with NaOH or sodium acetate. Records were obtained at pH values of 7.30, 7.00 and 6.50. One way Anova was used for statistical analysis.

Muscle tension decreased by nearly 17% at a lactate concentration of 20 mM at iso pH (pH=7.33), and lower pH values resulted in greater decreases in the genesis of muscle tension. However, at the same pH values, recorded muscle tension differed by approximately 14% between the high [La] and acetate treatments, and the effect of high [La] was more evident. High [La] resulted in greater decreases in the developing muscle tension if acidity was increased, but its molecular effects did not change significantly.

The inverse effects of 20 mM of lactate on the genesis of muscle tension at nearly iso pH values (pH=7.30-7.00) develop mainly via its molecular influences; on the other hand, its acidifying property predominates when acidity is increased (pH=6.50) but its molecular effects remain almost unchanged. Our findings may lead to the conclusion that acidic and molecular effects of high [La] at these pH values show an additive property and they influence the genesis of muscle tension in a negative manner.

Key Words: Lactate, acetate, pH, muscle tension development

Introduction

Fatigue can be defined as the decrease in the genesis of maximal muscle tension, and it may develop due to dysfunction of any critical site on the pathway from the central nervous system (CNS) to the actomyosin interaction. However, CNS is bypassed in the isolated nerve-muscle preparations, and the main factors leading to fatigue in these conditions can be listed as follows: disorders in the excitability of the muscle cell (1), decrease in calcium release from the sarcoplasmic reticulum (2,3) and metabolic inhibition of actomyosin complex formation (4-6). These factors may depend on the type of exercise (6), its duration, and the contribution of the types of contracting muscle fibers (2). In spite of extensive research on this issue, the real mechanism involved in fatigue has not been clarified yet.

When the optimal supply of oxygen is not available to the exercising muscle, pyruvate formed via glycolysis is converted to lactate. During intensive exercise, 25 mM of lactate may accumulate both in the exercising muscle and blood (7); this fact contributes to the decline in intramuscular pH in the "tired" fibers to a value of 6.00-6.30, from 7.00-7.20 in the resting state (5,8). This acidic shift in intramuscular pH leads to inhibition of

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glycolytic enzymes (9), ineffective excitation-contraction coupling (1,2,10-12), inhibition of calcium release from sarcoplasmic reticulum (1,13-15), competition of H^+ with calcium for binding to troponin (16,17), and direct inhibition of cross-bridges at the site of actin-myosin interaction (18). All these consequences lead to a decrease in muscle tension development. In light of these findings, lactate is considered to be one of the agents contributing to fatigue, acting via its acidifying property (pK value of lactic acid is 3.7; 19,20) (2,21).

In a study by Hogan *et al.* (1995), a high lactate concentration (high [La]) was shown to decrease muscle tension by 17% at iso pH in dog in situ gastrocnemius muscle (22), and this molecular effect of high [La] was demonstrated also in various other experimental models (skinned fiber, in vitro rat diaphragm muscle, isolated rat heart muscle) (7,23,24). In recent years, it was reported that high [La] exerts this H^+-independent effect without any change in muscle excitability (15), principally by decreasing calcium release from the sarcoplasmic reticulum (3,25,26); however, its minimal but significant effect on the calcium-activated force also plays a role (7).

Discrimination between the molecular and well-known H^+-dependent effects of high [La] that influence muscle tension negatively has been a matter of debate, along with the prominence of these effects with respect to changes in pH (15). In order to find an answer to these questions, we evaluated the effects of high [La] on the development of muscle tension at various pH values, to compare these effects with the developing tension when similar pH values were obtained by adding an inert reference acid (acetic acid), and consequently to determine the molecular and H^+-dependent effects of high [La].

Materials and Methods

Wistar strain albino rats (185-275 g) were used in this study. All procedures were approved by the Committee of Animal Care and Use, Çukurova University.

Muscle preparation: Following ether anesthesia, the entire diaphragm muscle was removed and placed into a dissecting chamber containing rat Ringer solution (in mM: 135 NaCl, 4 KCl, 1 $MgCl_2$, 1.3 Na_2HPO_4, 15 $NaHCO_3$, 11 glucose and 2.7 $CaCl_2$) continuously bubbled with 95% O_2 and 5% CO_2. A muscle strip from the ventral costal region was dissected while maintaining attachments at the central tendon and rib cage. The strip was fixed tightly to the suspending apparatus and transferred vertically into the experimental chamber for the measurement of developing tension. The central tendon part of the muscle strip was attached to the isometric force transducer (Harvard) for recording developing tension. The experimental chamber contained rat Ringer maintained at 30°C by Grand Instruments Type KA water circulator to maximize the isolated muscle function (27), and temperature was monitored by an EDT Instruments RE 357 temperature probe.

Experimental protocol and measurement of developing tension: Experiments were carried out at three different pH values (7.30, 7.00, 6.50). The pH of the solutions was adjusted either by adding 20 mM of L-(+)-Lactate (high [La]) (Sigma L-1875) or 5 mM of acetic acid (Act) (Sigma A-6283) (isoosmolarity was maintained by reducing NaCl content), which were buffered by NaOH or sodium acetate, respectively. Solution pH was measured continuously by using an EDT Instruments RE 357 pH probe. The experimental protocol was setup in similar conditions for the studied pH values (7.30, 7.00, 6.50), each of which consisted of control-high [La] and control-Act comparisons. It has been reported that high [La] (22,24) and acetate (28-31), applied extracellularly, result in intracellular acidification. Five mM of acetate did not exert any significant effect on the muscle tension at iso pH, and therefore it was used as the reference acid (Table).

Each muscle preparation was adjusted to its optimal length, at which maximal twitch was elicited. After 15 minutes of equilibration, muscle contractions (twitch) were elicited by supramaximal stimulation of the muscle with square wave impulses of 0.2 ms duration at 0.5 Hz. (Nihon Kohden SEN-3201 via stimulus isolator Nihon Kohden SS-102J). Developing tension was transferred via a coupler (Harvard coupler) to a Harvard Universal oscillograph. The contraction period for each treatment was 3 minutes. Every contraction bout was separated by 15 minutes of rest, which was sufficient for the L-(+)-Lactate isomer to reach equilibrium between intracellular and extracellular compartments (32).

Evaluation of Tension Development: Tension development was recorded continuously through the contraction bout and the results are given as normalized percentage values to eliminate individual variabilities among the muscle slices. In all treatments, the first

minute value of tension development was selected for evaluation. Normalization of the tension development was performed for C- high [La] and C- Act groups separately, at each pH value. During this procedure, the recorded value of tension development in the control solution was considered to be 100%, and changes in the tension development in high [La] or Act treatments were normalized for this value at each pH level.

Statistics: One way Anova was used for statistical analysis and 0.05 was taken as the level of significance. All values were expressed as the mean ± SEM.

Results

At iso pH (pH=7.30), 20 mM of lactate decreased muscle tension development by 16.4%, whereas 5 mM of acetate exerted no significant effect (Table, Figures 1A and B, Figure 2). However, when the solution pH was lowered by adding high [La] or acetate, recorded muscle tension decreased in both experimental groups (Table, Figures 1C-F, Figure 2). At similar pH values, recorded tension differed by nearly 14% between the high [La] and acetate treatments (Table, Figure 2). This difference contributed more to the inverse effect of high [La] on muscle tension development at pH 7.00 (elicited 14.14% of a total decline of 24.14%); however, when acidity was increased (pH 6.50), the difference remained the same, despite greater decreases in the recorded tension (at pH 6.50, tension development decreased by 28.5%, but the difference remained at 13.79%) (Table, Figure 2).

Discussion

The main results of the present study can be summarized as follows: the molecular, H^+-independent effect of 20 mM of lactate on the muscle tension development is more evident at nearly iso pH values (7.30-7.00), and when acidity is increased (pH 6.50) the molecular effect remains the same despite a greater decrease in tension. Thus it is possible to conclude that the molecular and acidic effects of high [La] influencing muscle tension development negatively may exhibit an additive property.

Effects of extracellular acidification on the intracellular pH: A membrane transport protein transports lactate along with H^+ across the cell membrane (32-35); consequently, when extracellular lactate is increased by experimental means, it not only leads to intracellular acidification but also exerts direct molecular effects (22,24). In studies involving various cell types, extracellularly applied acetate was also shown to result in intracellular acidification (28-31). When the effect of acetate on muscle tension development was investigated at iso pH, 5 mM of it was shown to exert no significant effect (Figure 1A, Table), and therefore it was used as the reference acid. Our results concerning the changes in muscle tension development in response to extracellular acidity (Table) are in accordance with those of Metzger and Fitts (1987), who obtained similar values (71%±2) in the rat diaphragm preparation when low-frequency stimuli were applied (8). In their study, intracellular pH was measured directly by ion-selective electrodes and

Table. Effects of acetate and high [La] on the muscle tension development at iso pH and low pH values (Mean±SEM).

pH	Acetate		High [La]	
	Tension %	pH	Tension %	pH
7.30	98.29±0.81	7.31±0.01	83.43±3.63[a]	7.33±0.03
	(n=7)		(n=7)	
7.00	90.0±2.21	6.99±0.01	75.86±3.73[a]	7.01±0.01
	(n=8)		(n=7)	
6.50	85.29±1.87[c]	6.52±0.01	71.5±3.16[ab]	6.50±0.01
	(n=8)		(n=8)	

a; significant when compared to acetate treatment at the same pH ($p<0.05$),

b; significant when compared to high [La] at pH 7.30,

c; significant when compared to acetate at pH 7.30.

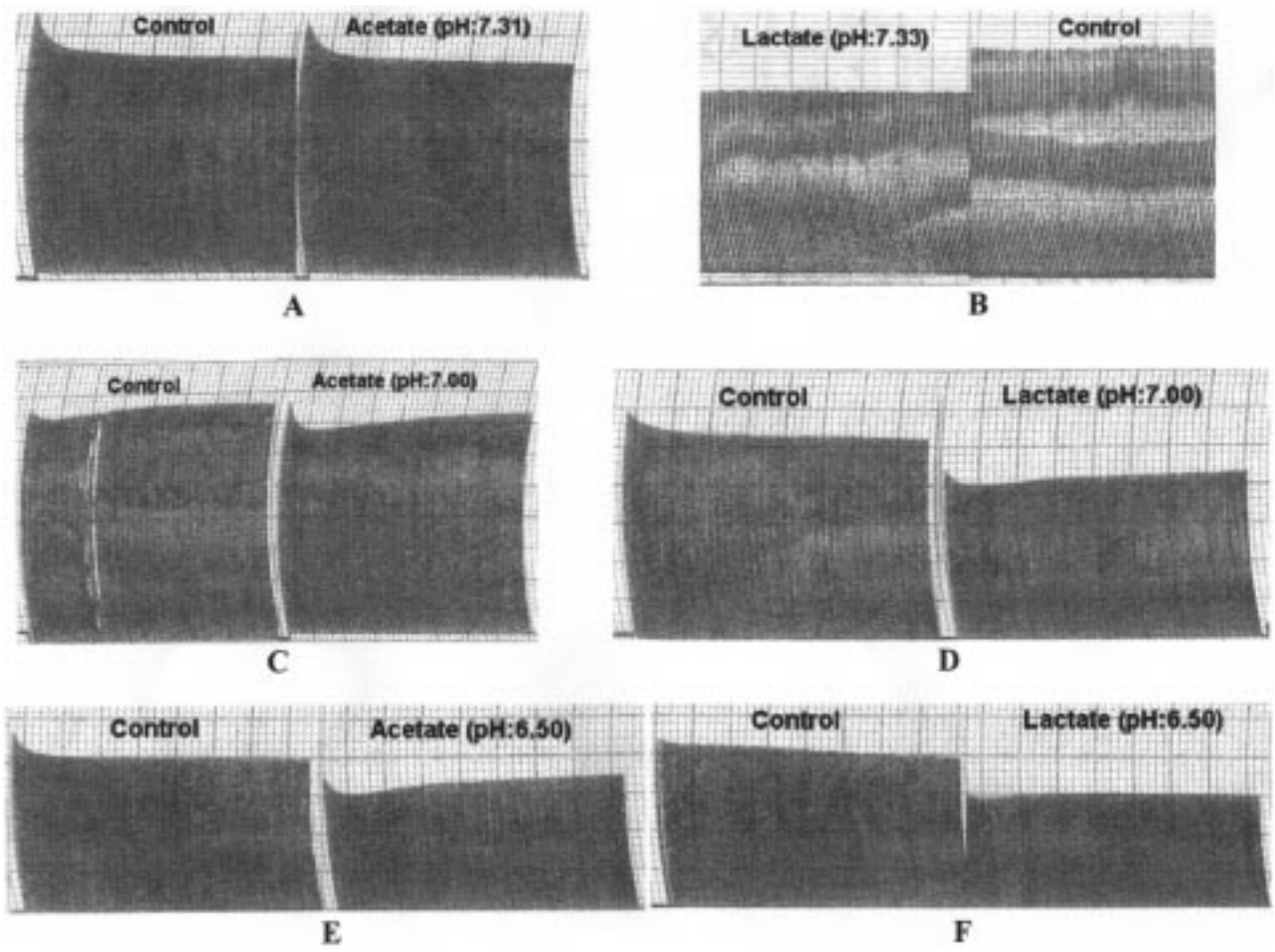

Figure 1. Illustration of records taken from A, acetate pH:7.30; B, high [La] pH:7.30; C, acetate pH: 7.00; D, high [La] pH: 7.00; E, acetate pH: 6.50; F, high [La] pH: 6.50.

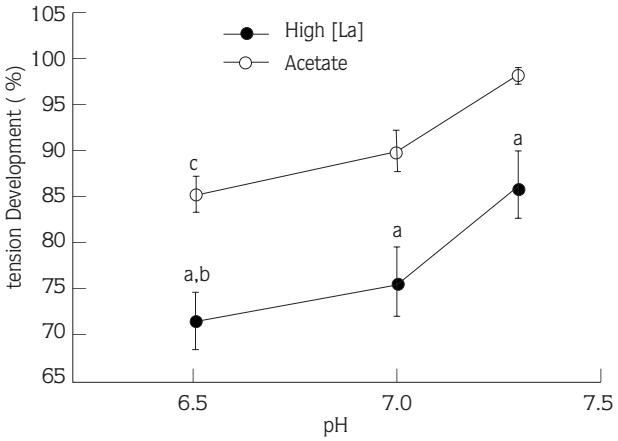

Figure 2. Effects of high [La] and acetate on the muscle tension development at various pH values (Mean±SEM). (a, b, c are significant when compared to acetate treatment at the same pH, to high [La] at pH 7.30, and to acetate at pH 7.30, respectively; $p<0.05$).

was shown to decline to 6.33 at this degree of fatigue. In another study using skinned vascular smooth muscle, tension recorded at pH 6.50 was reported to decline to 87% of the control (36). In light of these findings, it is possible to conclude that extracellular acidification, by also influencing the intracellular pH, may result in the changes in muscle tension development recorded in our study.

Molecular effects of high [La]: In the "tired" muscle, intracellular pH was determined to be as low as 6.00-6.30 (1,6,8,10,12,37,38). In our study, we also recorded significant decreases in the muscle tension development when pH was lowered by high [La] or acetate treatments. However, at the same pH, high [La] caused a greater decrease in the tension development than acetate did (Table, Figures 1A-F, Figure 2). A similar decrease in tension was recorded when dog in situ

gastrocnemius muscle was treated with 20 mM of lactate (22). On the other hand, in a study by Samaja *et al.* using isolated rat heart, solution pH was adjusted to 7.0 either by addition of lactate (20 mM) or by increased H^+ concentration, and both treatments resulted in similar decreases in muscle tension (80% of the control at pH 7.00) (24). However, this study was performed with heart muscle and records were obtained solely at pH 7.00. In a recent review concerning the muscle fatigue and release of calcium from the sarcoplasmic reticulum in skeletal muscle, 10-30 mM of lactate was reported to inhibit efficiently this calcium release, which is one of the most important steps in the mechanism of muscle contraction (26).

Conclusion

On the process of muscle tension development, the molecular effects of high [La] predominate at pH values of 7.30-7.00. However, when acidity is increased its H^+-dependent influences are of more importance, whereas its molecular effects remain almost unchanged. The nearly 14% greater decrease in the muscle tension development by high [La] at the same pH value, and the maintenance of this difference between lactate and acetate treatments at both pH levels, may be the consequences of lactate's molecular effects. It is possible to conclude that these two effects of high [La] (acidic and molecular) influencing the muscle tension in a negative manner, exhibit an additive property. However, further studies are needed to determine the mechanism of these molecular effects of high lactate by repeating the experiments at various other pH conditions.

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