Effect of lactate infusion on M-wave characteristics and force in the rat plantaris muscle during repeated stimulation in situ

Antony D. Karelis, Mariannick Marcil, François Péronnet, and Phillip F. Gardiner

Département de Kinésiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7

Submitted 13 January 2004; accepted in final form 5 February 2004

Karelis, Antony D., Mariannick Marcil, François Péronnet, and Phillip F. Gardiner. Effect of lactate infusion on M-wave characteristics and force in the rat plantaris muscle during repeated stimulation in situ. J Appl Physiol 96: 2133–2138, 2004.—It is unclear whether accumulation of lactate in skeletal muscle during exercise contributes to muscle fatigue. The purpose of the present study was to examine the effect of lactate infusion on muscle fatigue during prolonged indirect stimulation in situ. For this purpose, the plantaris muscle was electrically stimulated (50 Hz, for 200 ms, every 2.7 s, 5 V) in situ through the sciatic nerve to perform concentric contractions for 60 min while either saline or lactate was infused intravenously (8 rats/group). Lactate infusion (lactate concentration ~12 mM) attenuated the reduction in submaximal dynamic force (~49% vs. ~68% in rats infused with saline; P < 0.05). Maximum dynamic and isometric forces at the end of the period of stimulation were also higher (P < 0.05) in rats infused with lactate (3.8 ± 0.3 and 4.4 ± 0.3 N) compared with saline (3.1 ± 0.2 and 3.6 ± 0.2 N). The beneficial effect of lactate infusion on muscle force during prolonged stimulation was associated with a better maintenance of M-wave characteristics compared with control. In contrast, lactate infusion was not associated with any reduction in muscle glycogen utilization or with any reduction of fatigue at the neuromuscular junction (as assessed through maximal direct muscle stimulation: 200 Hz, 200 ms, 150 V).

Muscle force; performance; lactic acid; fatigue

IT HAS BEEN REPEATEDLY SUGGESTED that lactic acid accumulation in the muscle and in the extracellular fluid and/or a decrease in pH in response to exercise could be in part responsible for the development of muscle fatigue (see Refs. 3 and 13 for review). However, results from several studies that have examined the effect of lactate administration on muscle function in various experimental models do not consistently support this hypothesis (1, 2, 4, 5, 8, 11, 12, 18, 22, 23, 25–28, 30). Favero et al. (12) showed a large reduction in Ca2+ release from isolated sarcoplasmic reticulum vesicles incubated with lactate (37% decrease at 20 mM lactate), but in mechanically skinned fibers this effect appears much smaller (<10% decrease at 30 mM lactate) (8) or even reversed (220% increase at 20 mM lactate) (2). Andrews and Nosek (2) also reported that lactate slightly reduced Ca2+ uptake by the sarcoplasmic reticulum in intact isolated muscle fibers (13% decrease over 15 s but no effect over 1 min with 20 mM lactate). Consistent data indicate no inhibition of the excitation-contraction coupling by lactate in skinned (1, 4, 27, 28) and in intact muscle fibers (22, 26, 30). As for the electrical properties of the sarcolemma, Erdogan et al. (11) showed that lactate (20 mM) does not modify resting membrane potential or the amplitude of the action potential in muscle strips of the rat diaphragm stimulated indirectly in vitro. Nielsen et al. (23) and Pedersen et al. (25) actually showed that, in intact muscle stimulated directly or indirectly in vitro, lactate (20 and 10 mM, respectively) could partly restore M-wave area previously deteriorated by increasing K+ concentration in the bath. Finally, the effect of lactate on force development appears variable. Phillips et al. (26), in the mouse soleus and extensor digitorum longus (EDL) muscles, and Coast et al. (5), in rat diaphragm muscle strip in vitro, did not observe any change in isometric force when lactate concentration was increased to 20 or 10 mM, respectively. In contrast, Erdogan et al. (11), in rat diaphragm muscle strips stimulated indirectly in vitro, and Hogan et al. (18), in dog gastrocnemius muscle stimulated indirectly in situ, showed that lactate (20 and 14 mM, respectively) significantly reduced isometric muscle force. However, Nielsen et al. (23), in the rat soleus muscle, and Pedersen et al. (25), in both the rat EDL and soleus muscles, stimulated directly or indirectly in vitro, showed that the reduction in isometric force due to increasing K+ concentration in the bath was totally (23) or partially (25) reversed when lactate concentration was increased to 20 and 10 mM, respectively.

Muscle fatigue has been associated with impairments of action potential generation and/or propagation along the muscle fiber membrane (6, 13, 14, 24). Therefore, the purpose of the present study was to further investigate the effect of lactate infusion on the electrical properties of the muscle fiber membrane and the development of muscle fatigue. On the basis of studies from Nielsen et al. (23) and Pedersen et al. (25), we hypothesized that the electrical properties of the muscle fiber membrane and the development of muscle fatigue would be better maintained with lactate infusion during prolonged indirect stimulation in situ. Submaximal dynamic force of the plantaris muscle along with changes in M-wave characteristics were measured in anaesthetized rat during prolonged nerve electrical stimulation in situ in a control situation and with lactate infusion.

METHODS

Animal care and anesthesia. Adult female Sprague-Dawley rats weighing ~250 g were obtained from Charles River (St. Constant, PQ, Canada). The animals were housed by pair in grid cages in a room maintained at 20–23°C and 25% relative humidity, with a 12:12-h light-dark cycle. The animals were provided with commercially available laboratory rat chow and water ad libitum from the time of reception until the day of the experiment. The care and treatments of the animals were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and theAnimal 

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: F. Péronnet, Université de Montréal, Dept. of Kinesiology, P.O. Box 6128 Centre-Ville, Montreal, Quebec, Canada H3C 3J7 (E-mail: Francois.Peronnet@umontreal.ca).

http://www.jap.org 8750-7587/04 $5.00 Copyright © 2004 the American Physiological Society
animals were conducted according to the directives of the Canadian Council on Animal Care. The animals were anesthetized by intraperitoneal injections of an initial dose of ketamine and xylazine (61.5 mg/kg ketamine and 7.7 mg/kg xylazine). Two supplemental doses were given in the middle of preparation (approximately minute 45) and immediately before the beginning of stimulation (12.3 mg/kg ketamine and 1.5 mg/kg xylazine ip) to maintain deep anesthesia.

Animal preparation. As previously described (19), the plantaris muscle of the left leg was surgically isolated from the other ankle extensors, which were denervated and tenotomized at the proximal end of their distal tendon to avoid separating the common tendon of the extensors. The plantaris muscle was chosen because it contains a mixture of motor units (16) and fiber types (7) and is resistant to fatigue during submaximal prolonged stimulation (16, 19, 20). The calcaneous was clipped, leaving a bone chip attached to the common tendon, and a silk ligature (2-0 thread) was firmly placed around the bone-tendon interface. The animal was placed in a prone position on a stereotaxic table, and it was stabilized by clamps fixed in the head, vertebral column, left knee, and left foot. The hindlimb skin flaps were used to prepare a pool that was filled with mineral oil kept at 36–37°C by recirculation through a thermostatically controlled bath. The rectal temperature was monitored throughout the experiment and kept at 36°C by using a heating pad. For the nerve- and muscle-evoked contractions, a bipolar stimulation electrode was positioned in contact with the sciatic nerve, and the plantaris tendon was attached to a lever arm of a muscle puller servomotor (Cambridge LR 305B, Aurora Scientific, Aurora, ON, Canada) with a silk ligature. Surface electromyograph was recorded by using a ball electrode mounted on a spring, which was placed in contact with the origin side of the plantaris muscle, with the ground electrode placed through the gastrocnemius muscle.

The animals were divided randomly into two groups of eight animals and studied immediately before, during, and after a 60-min period of electrical stimulation of the sciatic nerve with a continuous infusion through a catheter in the left jugular vein of either saline alone (NaCl 0.9%) (7.25 ml/kg·h−1) or sodium l-(-)-lactate (sodium 2-hydroxypropionate) (Sigma Chemical) (0.96 g/kg·h−1), administered with saline 7.25 ml/kg·h−1 ip) to maintain deep anesthesia. Two supplemental doses were given in the middle of preparation (approximately minute 45) and immediately before the beginning of stimulation (12.3 mg/kg ketamine and 1.5 mg/kg xylazine ip) to maintain deep anesthesia. The infusion of lactate and the stimulation were both initiated at minute 0. The pattern of stretch (2 mm for 100 ms), these variables were measured in additional groups of rats infused with saline or with lactate as described in Experimental protocol. Both plantaris muscles were excised and frozen into liquid nitrogen immediately after the end of each experiment, and they were stored at −80°C until analysis. Muscle glycogen levels were measured by using the technique of Lo et al. (21).

Statistical analysis. Data are expressed as means ± SD. Comparisons were made by using a two-way ANOVA. When significant differences were revealed, a Scheffé’s post hoc test was performed. The level of statistical significance was set at \( P < 0.05 \).

RESULTS

Plasma glucose (Fig. 1) and insulin (Table 1) concentrations remained unchanged when saline or lactate was infused. However, plasma lactate concentrations, which remained unchanged when saline was infused, significantly increased in response to lactate infusion (Fig. 1). The 60-min period of stimulation significantly decreased muscle glycogen concentrations with no significant difference in the initial and final values between the two groups (Table 1). Blood pH did not significantly change in response to electrical stimulation of the plantaris muscle with infusion of saline or lactate (Table 1).

Submaximal dynamic force significantly decreased over the first 5 min of stimulation in the two groups and remained stable thereafter (Fig. 2). However, the reduction was significantly smaller in rats infused with lactate compared with control at minute 60 (−49 vs. −68%, respectively).

Twitch force significantly decreased after the 60-min period of stimulation with no significant difference between the two groups, whereas twitch half-relaxation time was not significantly modified (Table 1).
Figure 3 shows the maximum dynamic force before and after the 60-min period of stimulation. Maximum dynamic force significantly decreased in the control group, but it remained unchanged in rats infused with lactate for 60 min.

The maximum isometric force developed under indirect stimulation at the end of the 60-min period of stimulation was significantly higher in rats infused with lactate for 60 min than in the control group (Fig. 4). Direct stimulation increased muscle force ~20% in both groups.

Figure 5 shows changes in M-wave peak-to-peak amplitude, duration, and total area during the 60-min period of stimulation. When saline was infused throughout the experiment, a significant reduction in M-wave peak-to-peak amplitude and total area as well as an increase in duration were observed. When lactate was infused throughout the experiment, the reduction in M-wave peak-to-peak amplitude and the increase in duration were significantly lower, and no change in total area was observed.

DISCUSSION

Results from the present study show that muscle force significantly decreased by 68% of initial value in the control group during the 60-min period of stimulation. This marked reduction in submaximal dynamic force was associated with an increase in M-wave duration and with a decrease in M-wave peak-to-peak amplitude and total area. These observations confirm that muscle fatigue, in this model, is at least partly due to impairments of action potential generation and/or propagation along the muscle fiber membrane (9, 10, 15, 16).

We have recently shown that glucose infusion helped maintain the electrical properties of the muscle fiber membrane and attenuated fatigue in the rat plantaris muscle stimulated indi-
reflecting the maximal contractile capacity of the muscle, twitch characteristics are determined by sarcoplasmic reticulum function to a greater extent than are the other measures, it may indicate sarcoplasmic function is not implicated in the attenuating effects of lactate infusion on fatigue.

The observation that lactate alleviates the deterioration of the electrical properties of the muscle fiber membrane and attenuates muscle fatigue during prolonged electrical stimulation is in line with results from Nielsen et al. (23) indicating that lactate has a protective effect on muscle force production and M-wave area. In that study, tetanic force was reduced by 75% when isolated rat soleus muscles were incubated at a concentration of extracellular K⁺ of 11 mM. Increasing lactate concentration to 20 mM completely restored tetanic force. Furthermore, M-wave area was reduced when the muscle was exposed to high extracellular K⁺, but it almost completely recovered with administration of 20 mM lactate. In a subsequent study, Pedersen et al. (25) also showed that 10 mM lactate partially restored muscle force in EDL and soleus muscle incubated in 11 mM K⁺. In contrast, Hogan et al. (18) and Erdogan et al. (11) showed that lactate administration in a crossover design resulted in a reduction in muscle performance. In these studies, isometric force was significantly reduced when plasma lactate concentration was increased to 14 mM (18) or 20 mM (11) in the dog gastrocnemius muscle stimulated submaximally in situ through the sciatic nerve at 2 Hz and in rat diaphragm muscle strip stimulated supramaximally in vitro through the phrenic nerve, respectively. Results from the studies by Hogan et al. (18) and Erdogan et al. (11), on one hand, as well as Nielsen et al. (23) and Pedersen et al. (25), on the other hand, are difficult to compare, because fatigue was induced by different methods: prolonged electrical stimulation (11, 18) vs. large increase in K⁺ concentration (23, 25). As for the differences between the present study and those of Hogan et al. (18) and Erdogan et al. (11), they could be due to difference in the muscle studied [diaphragm (11) and gas-
tronemius (18) vs. plantaris in the present experiment), difference in the plasma lactate concentration achieved [20 mM (11) vs. ∼12 mM in the present experiment], and/or difference in the pattern of stimulation and the fatigue induced [low-frequency fatigue (11, 18) vs. high-frequency fatigue in the present experiment).

Taken together, data in the literature as well as data from the present experiment, do not consistently support the hypothesis that increase in lactate concentration is associated with muscle fatigue. On the contrary, as shown by Nielsen et al. (23) and Pedersen et al. (25), as well as in the present experiment, lactate could attenuate muscle fatigue. However, the mechanism(s) underlying this phenomenon remain(s) to be determined. In the studies by Nielsen et al. (23) and Pedersen et al. (25), both direct and indirect muscle stimulation were performed, but no comparison was made between the forces evoked in these two modes of stimulation. In the present experiment, maximal isometric force was compared at the end of the 60-min period of stimulation when the motor nerve or the muscle was stimulated. In rats infused with saline for 60 min but without stimulation of the nerve muscle preparation, the maximal isometric force produced was similar with direct and indirect stimulation. The maximal isometric force evoked by the indirect stimulation was significantly lower by 26% after the 60-min period of stimulation when saline was infused, but it was only 15% lower when lactate was infused. However, direct stimulation of the muscle significantly increased the maximal isometric force developed by ~20%, with no significant difference in the two groups. Accordingly, the protective effect of lactate infusion on force production during prolonged stimulation does not appear to be due to an attenuation of fatigue at the neuromuscular junction. In addition, as observed in our previous experiments with glucose infusion (19, 20), lactate infusion did not modify muscle glycogen utilization, which was similar in the two groups.

As discussed by Nielsen et al. (23), the beneficial effect of lactate on muscle performance could be due to the associated reduction in pH. In that study, lactate significantly decreased intracellular pH from 7.28 to 6.89. The authors concluded that acidification could counteract the depressing effects of elevated extracellular K+ concentration on muscle excitability and force, and they suggested that this could be due to a reduced inactivation of Na+ channels. Intracellular pH was not measured in the present study; however, as observed in other studies (11, 18), extracellular pH showed no changes with lactate infusion. Lactate could also modify Ca2+ handling by the sarcoplasmic reticulum. Nielsen et al. (23) did not report any effect of lactate administration on Ca2+ influx and total Ca2+ content of the muscles. However, Posterino and Fryer (28) observed a small increase in the rate of Ca2+ release from the sarcoplasmic reticulum of EDL fibers in presence of lactate, whereas Posterino et al. (27) showed that the rate of relaxation of the tetanic response was faster in the presence of lactate. It could also be suggested that changes in the osmolarity and/or Na+ concentration of the fluid surrounding the muscle fibers, due to sodium lactate infusion, could help maintain the electrical properties of the membrane and muscle performance. The infused lactate could also be a fuel for aerobic metabolism in the contracting muscle. Finally, it has been shown that an antioxidant supplementation in animals improved muscle performance (29), and Groussard et al. (17) showed that lactate could act as an antioxidant, able to scavenge both the hydroxyl superoxide anion radicals.

ACKNOWLEDGMENTS

The authors thank Gérard Ouëllet and Paul Martin for technical assistance.

GRANTS

This work was supported by a grant from the Natural Science and Engineering Research Council of Canada.

REFERENCES


J Appl Physiol • VOL. 96 • JUNE 2004 • www.jap.org


