Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous arm exercise

Nikolai Nordsborg,1 Magni Mohr,1 Lasse Dannemann Pedersen,1 Jens Jung Nielsen,1 Henning Langberg,2 and Jens Bangsbo1
1Copenhagen Muscle Research Center, Institute of Exercise and Sport Sciences, University of Copenhagen, 2100 Copenhagen; and 2Sports Medicine Research Unit, Copenhagen University Hospital, 2400 Copenhagen, Denmark

Submitted 21 January 2003; accepted in final form 21 March 2003

Nordsborg, Nikolai, Magni Mohr, Lasse Dannemann Pedersen, Jens Jung Nielsen, Henning Langberg, and Jens Bangsbo. Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous arm exercise. Am J Physiol Regul Integr Comp Physiol 285: R143–R148, 2003. First published March 27, 2003; 10.1152/ajpregu.00029.2003.—Interstitial K+ ([K+]i) was measured in human skeletal muscle by microdialysis during exhaustive leg exercise, with (AL) and without (L) previous intense arm exercise. In addition, the reproducibility of the [K+]i determinations was examined. Possible microdialysis-induced rupture of the sarcolemma was assessed by measurement of carnosine in the dialysate, because carnosine is only expected to be found intracellularly. Changes in [K+]i could be reproduced, when exhaustive leg exercise was performed on two different days, with a between-day difference of −0.5 mM at rest and 1.5 mM at exhaustion. The time to exhaustion was shorter in AL than in L (2.7 ± 0.3 vs. 4.0 ± 0.3 min; P < 0.05). Furthermore, [K+]i was higher from 0 to 1.5 min of the intense leg exercise period in AL compared with L (9.2 ± 0.7 vs. 6.4 ± 0.9 mM; P < 0.001) and at exhaustion (11.9 ± 0.5 vs. 10.3 ± 0.6 mM; P < 0.05). The dialysate content of carnosine was elevated by exercise, but low-intensity exercise resulted in higher dialysate carnosine concentrations than subsequent intense exercise. Furthermore, no relationship was found between carnosine concentrations and [K+]i. Thus the present data suggest that microdialysis can be used to determine muscle [K+]i kinetics during intense exercise, when low-intensity exercise is performed before the intense exercise. The high [K+]i levels reached at exhaustion can be expected to cause fatigue, which is supported by the finding that a faster accumulation of interstitial K+, induced by prior arm exercise, was associated with a reduced time to fatigue.

IN VIVO STUDIES have shown that potassium is lost from human skeletal muscle during intense exercise (5, 23, 28). After intense exercise venous plasma potassium concentrations ([K+]v), ranging from 6.5 to 8.5 mM have been reported (26). The concurrent accumulation of potassium in the muscle interstitium (21) has been suggested to be one cause of fatigue during intense exercise in humans (10). This hypothesis is supported by the finding of the same [K+]v at exhaustion when subjects performed two exhaustive exercise bouts on the same day, even though time to fatigue was different (3, 4). Recently, Juel et al. (21) studied interstitial potassium concentrations ([K+]i) during graded dynamic exercise in human skeletal muscle using the microdialysis technique. In that study, [K+]i was occasionally determined to be >10 mM. Extracellular K+ concentrations at this level have been shown to reduce peak tetanic force development by 25–75% in vitro studies (6, 19). However, no studies have determined the maximal attainable [K+]i in human skeletal muscle during dynamic intense exhaustive exercise.

During intense exercise, K+ is lost from the muscle cell through delayed rectifier K+ channels, but potassium may also be lost due to increased opening probability of the ATP-sensitive K+ (KATP) channels as a result of intracellular acidification (8, 9). This notion is supported by the results of a study in which subjects performed exhaustive one-legged knee-extension exercise with and without prior intense intermittent arm exercise (5). As a result of the arm exercise, time to exhaustion was reduced, and blood lactate and blood H+ concentrations were elevated before the exhaustive leg exercise. At exhaustion, intramuscular pH in the leg was lower when arm exercise was performed before the leg exercise. Furthermore, when corrected for exercise time, total potassium release to the blood was increased. Thus it is possible that intracellular acidification plays a role for potassium release in vivo. However, the effect of intracellular acidification on [K+]v has not been examined.

Thus the aim of the present study was to measure human muscle [K+]v during intense exercise to exhaustion and to test the hypothesis that a greater intracellular acidification leads to a higher rate of accumulation of potassium in the interstitium during exercise.

METHODS

Subjects. Six habitual active male subjects (age 24 ± 1 yr, height 183 ± 2 cm, body mass 74 ± 3 kg) participated in the study. The subjects’ maximal oxygen uptakes were determined to be 4.1 ± 0.2 l/min by an incremental bicycle test

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.
(17). The mass of the quadriceps femoris muscles was estimated to be 2.9 ± 0.2 kg based on Simpson’s rule as described by Ref. 18 and corrected based on a comparison between the anthropometrical measurements and CAT-scan determinations (ratio 1:0.80). The subjects were informed of any risks associated with the experiment before giving their consent to participate. The study was approved by the local ethics committee according to the code of Ethics of the World Medical Association (Declaration of Helsinki).

**Preexperimental procedure.** Leg exercise was carried out on a modified Krogh ergometer, permitting the exercise to be confined to the quadriceps femoris muscle (2). To familiarize the subjects with the exercise ergometer and to determine individual workloads resulting in fatigue after 3–5 min of exercise, all the subjects completed five to seven preexperimental sessions. During the preexperimental sessions the subjects were trained to keep a constant kicking frequency of 60 kicks/min (by visual feedback from a display showing the frequency) and to confine the exercise to the quadriceps femoris muscle (by verbal feedback based on online forcerecordings and electromyogram signals from the hamstring muscles). The subjects exercised until the point of fatigue with the objective determinant for terminating the exercise being a drop in kicking frequency to 55 kicks/min. During the preexperimental days the subjects were also familiarized to an arm cranking ergometer as described previously by Bangsbo et al. (5).

**Experimental procedure.** On the experimental days the subjects reported to the laboratory in the morning after consuming a light meal. Intake of caffeine on the day of the experiment and heavy physical activity on the day before the experiment were avoided. Six microdialysis probes were inserted parallel to the muscle fibers of the vastus lateralis muscle in the experimental leg under local anesthesia (lidocaine, 1 ml of 20 mg/ml) as previously described (21). Three of the probes were homemade (length of microdialysis membrane 30–40 mm, OD 0.22 mm) as described by Radegran et al. (24), whereas three of the probes (length of microdialysis membrane 30 mm, OD 0.6 mm) were commercially fabricated (CMA60; CMA Microdialysis). The perfusate was Ringer acetate containing (in mM) 130 Na⁺, 2 Ca²⁺, 4 K⁺, 1 Mg²⁺ and 30 Ac⁻. The exact perfusate K⁺ concentration was determined in each experiment. In addition, the perfusate contained 1 mM of lactate, 3 mM of glucose and 201Tl (activity <7,000 Bq/ml). After insertion, the probes were flushed and connected to a pump (CMA 102). Then they were perfused at a rate of 2 μl/min for 1 h, and then the perfusion rate was increased to 5 μl/min for the remaining part of the experiment.

**Exercise protocol.** Three experimental days were completed. Two of the experimental days were identical (L₁ and L₂), and on the third day (AL) an arm exercise period was added to the protocol (Fig. 1). The following procedure was common to all three days. After insertion of the microdialysis probes, the subjects rested for at least 60 min. This was followed by submaximal one-legged kicking at 20 W for two periods of 10 min separated by 15 min of passive rest. Dialysate was collected from the probes at rest and at two time intervals (2–5 and 5–10 min) during the 20-W exercise bouts. These two submaximal exercise periods had proved to improve the reproducibility of [K⁺]i determinations in pilot studies. After resting for an additional 90 min, a 10-min 10-W exercise period was completed. Dialysate was collected twice during the 10-W period (2–5 and 5–10 min.). Then the subjects rested for 18 min before performing exhaustive exercise at a power output of 62.8 ± 3.0 W. Dialysate was collected from 12 to 17 min during the resting period.

During the exhaustive exercise the first collection of dialysate was made from 0.5 to 2.0 min, representing the time interval from 0.0 to 1.5 min, when taking into account the delay due to the outlet tubing. Thereafter dialysate was collected in 1.5-min intervals with the duration of the last collection varying according to the time of exhaustion.

On day AL, the subjects performed arm cranking between the 10-W exercise period and the intense leg exercise (Fig. 1). The subjects performed the arm exercise (frequency 1 Hz) in an upright position at an intensity of 3.0 W. Dialysate was collected in each experiment. In addition, the perfusate contained 1 mM of lactate, 3 mM of glucose and 201Tl (activity <7,000 Bq/ml). After insertion, the probes were flushed and connected to a pump (CMA 102). Then they were perfused at a rate of 2 μl/min for 1 h, and then the perfusion rate was increased to 5 μl/min for the remaining part of the experiment.

**Exercise protocol.** Three experimental days were completed. Two of the experimental days were identical (L₁ and L₂), and on the third day (AL) an arm exercise period was added to the protocol (Fig. 1). The following procedure was common to all three days. After insertion of the microdialysis probes, the subjects rested for at least 60 min. This was followed by submaximal one-legged kicking at 20 W for two periods of 10 min separated by 15 min of passive rest. Dialysate was collected from the probes at rest and at two time intervals (2–5 and 5–10 min) during the 20-W exercise bouts. These two submaximal exercise periods had proved to improve the reproducibility of [K⁺]i determinations in pilot studies. After resting for an additional 90 min, a 10-min 10-W exercise period was completed. Dialysate was collected twice during the 10-W period (2–5 and 5–10 min.). Then the subjects rested for 18 min before performing exhaustive exercise at a power output of 62.8 ± 3.0 W. Dialysate was collected from 12 to 17 min during the resting period.

During the exhaustive exercise the first collection of dialysate was made from 0.5 to 2.0 min, representing the time interval from 0.0 to 1.5 min, when taking into account the delay due to the outlet tubing. Thereafter dialysate was collected in 1.5-min intervals with the duration of the last collection varying according to the time of exhaustion.

On day AL, the subjects performed arm cranking between the 10-W exercise period and the intense leg exercise (Fig. 1). The subjects performed the arm exercise (frequency 1 Hz) in an upright position at an intensity of ~140 W. The arm exercise started 1 min after the end of the 10-W period. It consisted of four 1-min exercise periods and one 1.5-min period separated by 0.5-min recovery periods. This was followed by 4.5 min of rest and another 1-min arm exercise period. Finally, the subjects rested 4 min before the intense exhaustive leg exercise was initiated. A similar arm cranking protocol has been shown to raise muscle and blood lactate

![Fig. 1. Illustration of the protocol. On 2 occasions (L₁ and L₂) subjects performed submaximal leg exercise followed by high-intensity leg exercise (EX) until exhaustion. On a third occasion (AL) subjects performed intense intermittent arm exercise before the intense leg exercise. ■, Collection of dialysate for carnosine determination; ☎, collection of dialysate for potassium determination.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.01020.2003)
concentrations before the exhaustive leg exercise (5). The experiments L1, L2, and AL were performed in random order.

Carnosine experiment. To obtain information about the degree of sarcolemmal rupture caused by the microdialysis probes, dialysate carnosine concentrations were determined in three subjects who completed a protocol identical to L1 and L2. Two CMA60 probes were inserted in the vastus lateralis muscle of each subject, as just described. Dialysate was collected from 10 to 20 min and 60 to 70 min after insertion, 5.0 to 9.5 min during the first 20-W exercise, 80 to 90 min after the second 20-W exercise, and during the intense work period (Fig. 1).

Analysis. The perfusion rate of the individual microdialysis probes was determined throughout the experiment by weighing the sample tube before and after collection. Samples were excluded if the perfusion rate deviated by >30% from the target perfusion rate, or if there was any sign of hemoglobin in the dialysate. The K⁺ concentration of the samples was measured by flame photometry (FLM3, Radiometer) using lithium as internal standard. For each sample the ²⁰³Tl activity was determined in a Packard autogamma counter to determine thallium loss as previously described (13, 21). Carnosine was determined from the dialysate as performed by Gutierrez et al. (15) using an HPLC method described by Suliman et al. (27).

Calculations. The data from L1 and L2 were pooled and treated as obtained in one experiment denoted L. The relative loss (RL) of ²⁰³Tl was calculated as $RL_{21} = (\text{perfusate activity} - \text{dialysate activity})/\text{perfusate activity}$. $[\text{K}^+]_i$ was calculated from the dialysate potassium concentrations, assuming that fractional ²⁰³Tl loss from the perfusate was equal to the fractional gain of K⁺ to the perfusate (21): $[\text{K}^+]_i = \text{K}^+_{\text{perfusate}} + [(\text{K}^+_{\text{dialysate}} - \text{K}^+_{\text{perfusate}})/RL_{21}]$.

Statistics. Values are means ± SE. For each subject an average $[\text{K}^+]_i$ was calculated from the individual measurements from each probe. Mean $[\text{K}^+]_i$, and SE were determined from these individual means. Possible differences in time to fatigue were tested using a paired Student’s t-test. Possible differences in $[\text{K}^+]_i$ were tested using a two-way ANOVA for repeated measurements. In case of significant main effects, a Student-Newman-Keuls post hoc test was used to identify the points of difference. In the carnosine experiment, data from the two probes from each of the three subjects were treated as individual data points. Possible differences were analyzed by a one-way ANOVA for repeated measurements, and the Student-Newman-Keuls post hoc test was used to identify points of difference. Significance was set at the 0.05 level.

RESULTS

Performance. There was no difference in time to exhaustion between L1 and L2 (3.9 ± 0.3 vs. 4.2 ± 0.2 min). In AL, time to exhaustion was shorter ($P < 0.05$) than in L (2.7 ± 0.3 vs. 4.0 ± 0.3 min).

$[\text{K}^+]_i$. There were no detectable differences in the $[\text{K}^+]_i$ response between L1 and L2 (Fig. 2). The mean difference between $[\text{K}^+]_i$ determined during L1 and L2 at various time points was −0.6 ± 0.4 mM at rest, −0.3 ± 0.4 mM after warmup, 1.1 ± 0.7 mM during exercise, and 1.5 ± 2.2 mM at exhaustion. Individual differences in $[\text{K}^+]_i$ determined during L1 and L2 are depicted in Fig. 3.

No differences in $[\text{K}^+]_i$ between AL (4.9 ± 0.3 mM) and L (4.2 ± 0.2 mM) were observed at rest before the intense exercise. In the period from 0 to 1.5 min of the intense exercise, $[\text{K}^+]_i$ was higher ($P < 0.001$) in AL than in L (9.2 ± 0.7 and 6.4 ± 0.9 mM; Fig. 4). $[\text{K}^+]_i$ at exhaustion was also higher ($P < 0.05$) in AL compared with L (11.9 ± 0.5 vs. 10.3 ± 0.6 mM). The $[\text{K}^+]_i$ at exhaustion ranged from 8.8 to 13.7 mM between subjects, and a large variation was apparent for individual measurements. When two 20-W exercise periods separated by 15 min were performed (Fig. 5), a larger increase in $[\text{K}^+]_i$ was apparent in the first 20-W period compared with the second ($P < 0.001$).

Carnosine in dialysate. In the period from 10 to 20 min after insertion of the microdialysis probes, the carnosine concentration in the dialysate was higher ($P < 0.05$) than during the two following resting periods (Fig. 6). The dialysate carnosine concentration was 105 ± 14 μM during the first 20-W exercise period and lower (50 ± 11 μM; $P < 0.05$) during the intense exercise.
DISCUSSION

The major findings of the present study were that the measurements of $[K^+]_i$ were reproducible and that $[K^+]_i$ increased to levels high enough to induce muscular fatigue during intense exercise. Additionally, arm exercise performed before leg exercise induced a faster $[K^+]_i$ increase, which was associated with a reduced time to exhaustion, supporting the hypothesis that accumulating potassium in the muscle interstitium contributes to the development of muscle fatigue.

In vitro studies have provided substantial evidence indicating that membrane depolarization caused by accumulation of extracellular potassium leads to development of muscular fatigue (6, 19). We found $[K^+]_i$ at exhaustion to be ~11 mM, with individual values from 9 to 14 mM. These values correspond to previous reports on $[K^+]_i$ determined in human skeletal muscle during exercise (14). It should be noted that because $[K^+]_i$ was determined in 1.5-min intervals, the true peak $[K^+]_i$ may have been even higher. $[K^+]_i$ in the range found in the present study drastically reduces peak tetanic force in rat soleus muscle (6). Thus the $[K^+]_i$ found in the present study would depolarize rat skeletal muscle to between ~65 and ~50 mV (6). In the rat, a depolarization to ~60 mV at physiological temperatures greatly reduces the Na$^+$ current (25) due to slow inactivation of Na$^+$ channels, ultimately leading to inexcitability of the muscle cells. Thus it seems plausible that the exercise-induced elevation of $[K^+]_i$...
found in the present study results in reduced excitability of the active muscle fibers.

During the first 1.5 min of exercise, [K⁺]i increased more when leg exercise was preceded by arm exercise, whereas no difference was present in the increase from the first to the last measurement during exercise in L and AL. In accordance with this, Bangsbo et al. (5) found that the release of K⁺ from the working muscles to the bloodstream was higher in AL than L and more pronounced at the beginning of the exercise period. One possible explanation could be that a substantial drop in muscle pH is required before the open probability of the KATP channels increases (8). The increased extracellular lactate and H⁺ concentration in AL (5) probably caused a reduced efflux of H⁺ from the active muscle cells (20), leading to a more rapid drop in muscle pH in AL than in L. As the exercise period progresses, the difference in the intracellular H⁺ between AL and L was probably reduced (22), and thus the possible difference in potassium release via H⁺ activation of the KATP channels is likely to have been reduced.

The faster accumulation of K⁺ in AL was associated with a more rapid development of fatigue and supports the hypothesis that accumulation of K⁺ in the muscle interstitium is causatively linked to muscle fatigue (26). However, the finding of different [K⁺]i at exhaustion in AL and L indicates that K⁺ is not the only agent acting to induce muscular fatigue during intense exercise. It is likely that accumulation of extracellular K⁺, intracellular H⁺, and/or intracellular Pi in combination acts to induce fatigue (10, 29). It is also possible that other factors are causing the reduced intracellular calcium release observed during muscular fatigue (1).

At exhaustion a pronounced variation in [K⁺]i was observed. There may be several explanations for this finding. First, physiological spatial heterogeneity caused by varying degrees of muscle fiber activation in different regions of the muscle is one likely reason. Second, differences in the fiber type composition around the microdialysis probe could be a possibility, because different fiber types in the mouse are known to have different capacities for maintaining ion homeostasis (7). It should be noted, however, that differences in Na⁺-K⁺-ATPase density in relation to fiber types have not been demonstrated in humans.

The use of microdialysis allows determination of [K⁺]i in exercising human skeletal muscle (21). Determination of [K⁺]i has previously only been possible from calculations using several assumptions (16). Nevertheless, it should be discussed whether [K⁺]i determined by microdialysis can be considered valid. To investigate if rupture of the sarcolemma occurs when the microdialysis probes are inserted in human skeletal muscle, the dialysate was analyzed for carnosine concentrations. Carnosine is found in high concentrations inside the muscle cell (12) but is undetectable in blood plasma (15). High carnosine concentrations were observed in the dialysate during the first 20-W exercise period, indicating that rupture of the sarcolemma did occur. However, the low carnosine concentrations in the dialysate during the intense exercise period suggest that multiple exercise periods result in gradual reduction of sarcolemmal rupture. The higher dialysate carnosine concentrations during exercise compared with rest are probably not only due to a greater degree of sarcolemmal rupture, because the recovery of carnosine is most likely higher during exercise. No data exist on the recovery of carnosine, but the recovery for adenosine has been shown to change from ~35% at rest to ~60% during exercise (11). However, such an increase in recovery during exercise cannot explain the entire difference in the carnosine concentrations between rest and exercise. No relationship existed between [K⁺]i and dialysate carnosine concentrations (Fig. 7). This suggests that muscle rupture does not have a considerable effect on the examined concentrations of [K⁺]i. Taken together, the reproducibility of [K⁺]i in L1 and L2, the finding of a difference between AL and L, as well as the lack of correlation between [K⁺]i and carnosine, suggest that microdialysis can be used for determination of interstitial K⁺ kinetics in exercising human skeletal muscle.

In summary, the present study showed that muscle interstitial K⁺ kinetics can be determined by microdialysis, but we propose that the subjects perform a period of low-intensity exercise before measurement of [K⁺]i is made during exercise. This study showed that [K⁺]i reached levels during intense exercise that can be expected to cause fatigue. In support of this notion is the observation that the faster accumulation of interstitial K⁺ induced by prior arm exercise was associated with a reduced time to fatigue. However, an increase in [K⁺]i during intense exercise appears not to be the only cause of development of muscular fatigue during high-intensity exercise, because [K⁺]i at exhaustion was different when leg exercise was performed with or without previous arm exercise.

We greatly acknowledge the help with the analysis of carnosine concentrations provided by Dr. B. Anderstam from Department of Renal Medicine, Clinical Research Center (KFC), Stockholm, Sweden. This study was supported by grants from Team Danmark and Danish National Research Foundation Grant 504–14.

REFERENCES

6. Cairns SP, Flatman JA, and Clausen T. Relation between extracellular [K⁺], membrane potential and contraction in rat


