Wheel-running exercise alters rat diaphragm action potentials and their regulation by K+ channels

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Van Lunteren, Erik, and Michelle Moyer. Wheel-running exercise alters rat diaphragm action potentials and their regulation by K+ channels. J Appl Physiol 95: 602–610, 2003. First published April 18, 2003; 10.1152/japplphysiol.00711.2002.—Endurance exercise modifies regulatory systems that control skeletal muscle Na+ and K+ fluxes, in particular Na+-K+-ATPase-mediated transport of these ions. Na+ and K+ ion channels also play important roles in the regulation of ionic movements, specifically mediating Na+ influx and K+ efflux that occur during contractions resulting from action potential depolarization and repolarization. Whether exercise alters skeletal muscle electrophysiological properties controlled by these ion channels is unclear. The present study tested the hypothesis that endurance exercise modifies diaphragm action potential properties. Exercised rats spent 8 wk with free access to running wheels, and they were compared with sedentary rats living in conventional rodent housing. Diaphragm muscle was subsequently removed under anesthesia and studied in vitro. Resting membrane potential was not affected by endurance exercise. Muscle from exercised rats had a slower rate of action potential repolarization than that of sedentary animals (P = 0.0098), whereas rate of depolarization was similar in the two groups. The K+ channel blocker 3,4-diaminopyridine slowed action potential repolarization and increased action potential area of both exercised and sedentary muscle. However, these effects were significantly smaller in diaphragm from exercised than sedentary rats. These data indicate that voluntary running slows diaphragm action potential repolarization, most likely by modulating K+ channel number or function. 

Muscle; membrane potential; action potential; resting membrane potential; running

MUSCLE MEMBRANE POTENTIAL is determined by the relative concentrations of Na+, K+, and Cl− inside and outside the cell, as well as by the relative permeability of the cell membrane to these ions. At rest, muscle membrane is permeable predominantly to K+ and Cl− so that resting potential is close to the equilibrium potentials of these two ions. During an action potential, a sudden increase in Na+ permeability results in membrane depolarization and charge movement, with consequential release of Ca2+ from the sarcoplasmic reticulum, and, via a cascade of biochemical reactions, muscle contraction (35, 36). For a given resting potential, greater degrees of depolarization (up to membrane potential of ~0 to 20 mV) result in greater charge movement, augmented Ca2+ entry from the sarcoplasmic reticulum, and stronger contraction (4). The kinetics of the action potential also influence muscle contractile performance by altering the mechanically effective contractile period. Longer action potential durations enhance Ca2+ release from the sarcoplasmic reticulum and augment muscle contractile force (19, 22, 27, 39, 40). The speed with which the membrane repolarizes and hence the duration of the action potential are in turn determined to a large extent by the rapidity and degree to which K+ conductance increases (13).

Efflux of K+ from muscle during repetitive contractions leads to depolarization of resting membrane potential and a rise in serum K+ levels, especially during high-intensity exercise (25, 29, 33, 44). Endurance exercise diminishes K+ efflux during exercise in humans and animals (16, 18, 23, 24). This has been attributed to an upregulation of Na+-K+-ATPase concentration and/or activity (17, 18, 24). Of note, however, is that Kjeldsen et al. (16) found that training attenuated exercise-induced hyperkalemia without a change in Na+-K+-ATPase concentration. The latter finding could potentially be explained if training reduces K+ efflux through K+ channels. This should lead to a prolongation of action potential duration, in particular a slowing of action potential repolarization.

The effects of endurance exercise on skeletal muscle electrophysiological properties are not well defined. Studies have found variable effects of endurance exercise on resting membrane potential, with one investigation in dogs indicating hyperpolarization (18) and another report in rats indicating no change (7). Determination of whether exercise alters skeletal muscle action potential properties is important to better understand adaptive responses to endurance exercise. In addition, if exercise-induced alterations in action potential properties are in a direction that could improve muscle contractile function, this would strengthen the scientific rationale for the development of ion channel-based therapeutic approaches for skeletal muscle contractile dysfunction. The hypothesis of the present investigation is that endurance exercise slows diaphragm muscle action potential repolarization.

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The diaphragm was chosen over a limb muscle for the present study because our laboratory used the diaphragm in previous studies examining effects of K⁺ channel blockers on muscle contractile and electrophysiological properties (38–40) and because several animal studies have indicated that whole body endurance exercise (running) produces both physiological and biochemical changes in the diaphragm. For example, Gosselin et al. (11) found that treadmill running increased rat diaphragm succinate dehydrogenase activity in type I, IIa, and IIb fibers. Powers et al. (31) found that treadmill running increased succinate dehydrogenase activity in type I and IIa fibers, and increased capillary density in type I, IIa, and IIb fibers in rat diaphragm. In a separate study, Powers et al. (32) found that in treadmill running increased activities of 3-hydroxyacyl-CoA dehydrogenase, glutathione peroxidase, and citrate synthase in the diaphragm of young adult rats. Vrabas et al. (41) found that treadmill running improved rat diaphragm fatigue resistance, increased citrate synthase, augmented levels of superoxide dismutase, and increased the proportion of type I myosin heavy chain. Hughes and Gosselin (14) found that treadmill running resulted in rat diaphragm muscle being more resistant to the adverse effects of lengthening contractions.

METHODS

The studies were performed with male Sprague-Dawley rats. All protocols were approved by the Institutional Animal Care and Use Committee and conformed to animal care guidelines established by the National Institutes of Health. Voluntary wheel running was used as the exercise paradigm to avoid any physiological changes that may occur due to the stress of forced treadmill running (28). The animals were randomly divided into two groups: sedentary and exercise. Rats in the exercise group (n = 8) were housed singly in standard-size polycarbonate living chambers, which were modified to allow access to an immediately adjacent running wheel. The running wheel (circumference 1.13 m) was equipped with a magnetic counter, the output of which was sent to a computer, allowing quantification of the number of revolutions per day (Lafayette Instrument, Lafayette, IN). Sedentary animals (n = 8) lived in conventional standard-size polycarbonate rat housing. Both groups of animals were given ample access to food and water 24 h/day. The animals entered the study at an age of 8 wk and spent the subsequent 8 wk in either running wheel-equipped or standard housing.

After 8 wk of wheel running exercise or no running exercise, the animals (age now 16 wk) were anesthetized with intraperitoneal urethane (initial dose 1 g/kg, with supplemental amounts as needed). Rats were allowed access to the wheel (and continued to run regularly) until shortly before they were anesthetized, with at most 5–10 min elapsing for transport from the housing facility to the laboratory. Rats ran on and off throughout the day and night (albeit more at night than during the day, being nocturnal animals), rather than exercising for a single period of time once per day, and hence no attempt was made to differentiate between the effects of acute vs. chronic exercise. The diaphragm muscle was removed with intact rib and central tendon origins and insertions, keeping the phrenic nerve attached, and studied in vitro. Each hemidiaphragm had a small strip removed for contractile studies, with the remainder being used for action potential recordings. All data were derived from the costal diaphragm. Tissue dissections and both electrophysiological and contractile studies were performed in physiological solution consisting of the following (in mM): 135 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, 15 NaHCO₃, and 11 glucose, with the pH adjusted to 7.35–7.45 while being aerated with 95% O₂–5% CO₂.

For action potential recordings, diaphragm with attached phrenic nerve was pinned in a Sylgard-lined 35-mm petri dish. The temperature of the preparation was maintained at 37°C by using a Peltier heating device (Medical Systems, Greenvale, NY). Aerated solution flowed constantly from a reservoir through the petri dish. The solution was not aerated in the petri dish to minimize vibration, but it was verified to be well oxygenated by the use of a dissolved-oxygen meter (World Precision Instruments, Sarasota, FL). The phrenic nerve was placed in a suction electrode and stimulated with supramaximal voltages, using a pulse width of 0.2 ms. Intracellular recordings were made with glass microelectrodes (resistances of 5–15 MΩ when filled with 3 M KCl), which were fabricated with a Brown-Flaming micropipette puller (Sutter Instruments, Novato, CA). Resting membrane potential and action potentials were recorded with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). After determination of resting membrane potential, the phrenic nerve was stimulated at a low frequency (2 Hz) and the resulting action potential(s) recorded. Zero membrane potential was defined immediately before fiber impalement and confirmed as having been stable after electrode dislodgement from the fiber, which typically occurred after one or at most a few contractions. Electrophysiological signals were digitized, collected on-line (Axotape software, Axon Instruments, Foster City, CA), and stored on the hard drive of a computer for later analysis. Data analysis utilized a combination of the manually driven cursors provided with the Axotape software and the semiformalized analysis capabilities of the Strathclyde Electrophysiology Software Whole Cell Program (distributed by Dagan, Minneapolis, MN).

Small segments of data containing the action potentials were exported from Axotape to Whole Cell Program for the latter method of analysis. Action potential properties were characterized as follows: height (difference between resting membrane potential and the peak positive voltage), maximal rate of rise (most rapid rate of depolarization), 10–90% rise time (time required for the depolarizing phase to go from 10 to 90% of action potential height), 50% decay time (T 50%; time required for the action potential to repolarize 50% of the way from its peak back to resting membrane potential), and area (the integral of membrane potential during the action potential measured relative to resting membrane potential). Resting membrane potential and action potentials were analyzed for five fibers per hemidiaphragm before and a similar number of fibers per hemidiaphragm after addition of the K⁺ channel blocker (see below).

For contractile studies, strips of diaphragm muscle were mounted vertically in a double-jacketed chamber, the temperature of which was maintained at 37°C. A pair of platinum electrodes were placed parallel to the muscle strips, and supramaximal voltages were delivered by using a pulse width of 1 ms. The length of the muscle strips were adjusted to that at which twitch force was maximal (optimal length), and it was kept there for the remainder of the study. Twitch contractions were used to determine force per cross-sectional area, isometric contraction time, and isometric half-relaxation time. The cross-sectional area was calculated on the
basis of the following formula: cross-sectional area = (muscle mass)×(fiber length)−1×(muscle specific gravity)−3, assuming a muscle density of 1.06 g/ml. The force-frequency relationships were not measured because this would require multiple stimulations over a range of low to high frequencies, which could have led to force loss before the onset of fatigue testing. Distance running most likely acts more as an aerobic than an anaerobic form of muscle training and hence could potentially improve muscle fatigue resistance more than peak force. Fatigue resistance was tested during intermittent 20-Hz train stimulation, by using a train length of 0.33 s and one train per second. For the fatigue tests, force was normalized to twitch force at the onset of the study before any drug additions to factor out the effects of variability among strips in absolute force. The same approach has been used previously for studies of K+ channel blockers and muscle fatigue (38, 39).

To test whether K+ channels accounted for differences between muscles from exercised and sedentary animals, the K+ channel blocker 3,4-diaminopyridine (DAP) was added in a concentration of 0.3 mM. The concentration of DAP chosen was on the basis of a previous study noting near maximal increases in diaphragm twitch force with this amount (38), as was on the basis of a previous study noting near maximal force. The concentration of DAP chosen was tested by using separate muscle strips without and with the channel blocker.

K+ channels accounted for differences among rats of the animals ran on average 16 km/day, with a range among rats of 5–16 km/day. The body weight of the animals at the end of the no-exercise or exercise period was 546 ± 14 g for the sedentary rats and 468 ± 17 g for the exercising rats.

Effects of wheel running. There was no difference in diaphragm resting membrane potential between exercised and sedentary rats (−74.1 ± 0.8 mV for exercised animals, −72.5 ± 0.7 mV for sedentary animals). Diaphragm action potentials, however, were affected by running exercise. Specifically, action potential repolarization was significantly slower in the exercised than in the sedentary animals (P = 0.0098), as reflected by a prolongation in the time for the action potential to repolarize by 50% (T 50%) (Fig. 2). In contrast, action potential depolarization was not affected by exercise nor were there significant changes in action potential height or area.

Diaphragm twitch force normalized for cross-sectional area did not differ between exercised and sedentary animals nor was contraction time altered by exercise (Fig. 3). However, half-relaxation time was significantly slower in diaphragm from exercised compared with sedentary rats (P = 0.006). Diaphragm fatigue resistance during 20-Hz stimulation was similar in the
two groups of animals (Fig. 3), even when force loss was calculated relative to initial 20 Hz force.

Effects of K\(^{+}\) channel blockade. DAP did not alter diaphragm resting membrane potential of either sedentary or exercised rats (data not shown). In addition, action potential height and rate of depolarization remained unchanged with DAP (Fig. 4). However, action potential repolarization rate was slowed significantly,
and action potential area was increased significantly, by DAP (Fig. 5). Furthermore, the effects of DAP were smaller in muscle from exercised than sedentary animals. Thus in the absence of DAP action potential repolarization rate was significantly slower for muscle from exercised rats but in the presence of DAP repolarization was significantly faster for muscle from exercised than sedentary rats. Moreover, in the absence of DAP action potential area was similar for exercised and sedentary muscle, but in the presence of DAP action potential area was significantly less in exercised than sedentary muscle.

Twitch force was increased significantly by DAP, by 71.5 ± 12.3% for diaphragm from exercised rats and by 98.4 ± 7.4% for muscle from sedentary animals. There was a trend for DAP to augment diaphragm force to a lesser extent in exercised than sedentary rats ($P = 0.085$). Twitch isometric contraction time was prolonged significantly by DAP in both unexercised and endurance-exercised muscle, whereas there were no significant alterations in twitch half-relaxation time (Fig. 6). DAP also improved force over time during the course of fatigue-inducing 20-Hz stimulation for diaphragm muscle from both exercised and sedentary animals (Fig. 7). This was slightly more prominent for muscle from sedentary than exercised animals, but this only reached statistical significance at a single point (20 s) during the course of the fatigue trial.

**DISCUSSION**

The major findings of the present study were that 8 wk of voluntary endurance exercise (wheel running) slowed diaphragm action potential repolarization and that it attenuated the effects of the $K^+$ channel blocker DAP on action potential duration and area. This endurance exercise regimen was not associated with any changes in muscle force or fatigue resistance, but it did lead to a slowing of muscle relaxation during isometric twitch contractions.

Action potential depolarization results from the opening of $Na^+$ channels, whereas repolarization is due to both the closure of $Na^+$ channels and the opening of $K^+$ channels (13). The effects of exercise in slowing action potential repolarization are unlikely to be due to an alteration in $Na^+$ channel density or conductance or to an alteration in $Na^+$ channel activation kinetics. These changes should have altered the rate of action potential depolarization, which was not the case. An exercise-induced slowing of $Na^+$ channel
alterations in Na\(^+\) channels should have affected the rate of action potential depolarization, and alterations in Cl\(^-\) channels should have affected resting membrane potential.

Resting membrane potential was not altered significantly by exercise, in contrast to a previous study in dogs (18) but in agreement with a previous study in the rat (7). Skeletal muscle contains many types of K\(^+\) channels, each with different roles in the regulation of cellular electrophysiological function. Inward rectifier K\(^+\) channels contribute importantly to resting membrane potential, whereas delayed rectifier K\(^+\) channels play the predominant role in action potential repolarization (6, 22, 27, 39, 40). Other K\(^+\) channel types (e.g., ATP-sensitive K\(^+\) channels and Ca\(^{2+}\)-activated K\(^+\) channels) appear to have minor electrophysiological roles in relatively quiescent muscle (5, 21, 40) but could conceivably be more important in actively contracting muscle. (Direct testing of this is not feasible with the methodology used in the present study, because repetitive contractions at moderate- to high-stimulation frequencies invariably dislodge the intracellular electrodes.) The present data, which indicate a slowing of action potential repolarization but no change in resting membrane potential with endurance exercise, suggest that delayed rectifier K\(^+\) channels are more likely than inward rectifier K\(^+\) channels to have been altered by the running program, but this needs to be tested directly in future studies.

The effects of exercise on K\(^+\) channel regulation of muscle contraction have been demonstrated previously in vascular smooth muscle. Bowles et al. (3) examined coronary artery tone after 16–20 wk of treadmill running in miniature swine. Several K\(^+\) channel blockers (4-aminopyridine, tetraethylammonium, iberiotoxin) ...
all increased resting coronary artery tension, but this was consistently greater in vascular muscle from exercised than sedentary animals. Other studies have noted that endurance exercise prolongs cardiac muscle action potential duration (12, 15, 37), which appears to be due in part to alterations of specific K⁺ currents (15). The present data extend these findings by indicating that regulation of K⁺ channel function by exercise is not unique to cardiac muscle and cardiac vascular smooth muscle.

Several studies have examined effects of endurance exercise on the contractile properties of the diaphragm. Metzger and Fitts (26) noted in rats that 6 wk of high-intensity treadmill exercise did not alter diaphragm specific force, maximal shortening velocity, or fatigue resistance but that it did increase the rate of fall of twitch tension. More recently Dupont-Versteegden et al. (8) found in mice that 9–12 mo of voluntary wheel running did not alter diaphragm force per cross-sectional area, optimal length, isometric contraction or half-relaxation times, twitch-to-tetanic ratio, or fatigue resistance. Finally, Vrabas et al. (41) found in rats that 10 wk of treadmill running improved diaphragm fatigue resistance but had no effects on specific twitch and tetanic forces, rate of force development, isometric half-relaxation time, twitch-to-tetanic force ratio, or maximal shortening velocity. The present study concurs with these studies that the contractile changes resulting from endurance exercise are relatively modest, despite previously described biochemical alterations with endurance exercise, such as increased oxidative mitochondrial enzyme levels, elevated antioxidant enzyme levels, altered myosin subtype distributions, and augmented Na⁺-K⁺-ATPase activity (11, 17, 18, 24, 30, 31, 34, 41). It is possible that under greater degrees of metabolic stress, endurance exercise might convey a more positive functional adaptation.

There are several possible explanations for differences among these studies and the present one in the extent to which endurance exercise altered diaphragm contractile properties. First, there are differences between forced running (previous studies) and voluntary running (present study), in that the former adds a type of stress not present with the latter. This was discussed recently by Moraska et al. (28), who noted that forced treadmill running resulted in the potentially negative adaptations of adrenal hypertrophy, thymic involution, decreased serum cortisol-binding globulin, elevated lymphocyte nitrite concentrations, suppressed lymphocyte proliferation, and suppressed antigen-specific IgM, which are changes commonly associated with stress. Alterations in the neuroendocrine axis could potentially have had secondary effects on muscle properties. Second, the distance run per week was longer in the present compared with previous studies. Hughes and Gosselin (14) had their (young) animals run 27 m/min, 45 min/day, 5 days/wk by the end of the training period; this amounts to 6,075 m/wk. Vrabas et al. (41) did not specify the exact mileage for their animals, but they stated the animals ran for 60 min/day and then referenced the protocol of Metzger and Fitts (26), which used 40 m/min, 27 min/day, 5 day/wk, or 5,400 m/wk (so even if doubled this amounts to 10,800 m/wk). In the present study, during the last several weeks the animals ran ~10,000 m/day or ~70,000 m/wk, a weekly distance that is 10-fold longer than in the Hughes and Gosselin (14) study. Third, the Hughes and Gosselin study used a 15% grade, and it would appear that Vrabas et al. (41) did as well, whereas the grade is hard to quantify with wheel running (but is probably less). Fourth, in Hughes and Gosselin (14) study animals exercised continuously for 45 min, and in the Vrabas et al. (41) study the exercise was accomplished over the course of 60 min, whereas in the present study the exercise was intermittent but spread out over the course of the animals’ waking hours.

The present study focused on a single K⁺ channel blocker several reasons. First is that studies using a single agent (DAP) required over 20 wk to carry out because of the need for two groups of rats being housed in the running wheel-equipped cages (2 groups of 4 animals each for 8 wk), followed thereafter by data analysis. Testing the effects of an additional five or more drugs would take an additional 2 yr or so and increase the amount of data sixfold, which is a time frame and amount of data more appropriate to a series of studies. Second, action potential repolarization in rat diaphragm appears to be due predominantly to delayed rectifier K⁺ channels, as expected on the basis of what is known about the functional roles of the various types of K⁺ channels. For example, in previous studies (40), our laboratory found that apamin and charybdotoxin (which similar to iberiotoxin block Ca²⁺-activated K⁺ channels) had no effects on action potentials, whereas glibenclamide (which blocks ATP-sensitive K⁺ channels) slowed action potential repolarization to a very small extent (12% prolongation of 50% decay time compared with 298% prolongation with DAP and 183% prolongation with 4-aminopyridine). It is possible that endurance exercise results in the participation of K⁺ channels subtypes in action potential repolarization not normally seen in muscle from sedentary animals, but the likelihood is not high enough to delay communication of the present results. Similarly, voltage clamp studies would also be of interest to further characterize delayed rectifier (and possibly other) K⁺ channel opening time, conductance, and density, but this too is beyond the scope of the original hypothesis.

In the present study, resting membrane potential and action potentials were analyzed for five fibers per hemidiaphragm. We did not pool the data for each hemidiaphragm, similar to previous studies (39, 40). The diaphragm is composed of a heterogeneous population of fiber types, and it was therefore felt important to ensure that heterogeneity among fibers of a given diaphragm sample would be reflected in the SE values and the statistical analysis. (Use of pooled data did not change the overall findings, however.) On the basis of known motor unit behavior of respiratory muscles,
when breathing is stimulated motor units previously quiescent are recruited, and active motor units increase their firing frequency. Thus there potentially may have been differences among fibers in the extent to which they increased their activity with exercise. Gosselin et al. (11) found that treadmill running increased succinate dehydrogenase activity in type I, type IIa, and type IIb fibers of the rat diaphragm, suggesting that in fact motor units with a wide range of recruitment thresholds are all activated during training. The present study did not note a binomial distribution in action potential properties among fibers, but this may not be very meaningful because there was no way of knowing the previous activation history of the fibers and no way of ensuring that recordings were made from the full spectrum of fibers with different activation histories.

A technical issue is whether contraction artifacts interfered with the recordings of action potentials. Although the muscles were pinned down, this did not completely prevent fiber shortening during contraction. Movement artifacts were generally not a problem for a single action potential, as depicted in the action potential example figures (Fig. 2A). Any action potentials in which contraction artifacts were present were discarded from analysis. The reasons for the lack of contraction artifacts positing a substantial problem for single action potentials are as follows. First, the action potential precedes contraction, because it is the depolarization that leads to Ca$^{2+}$ influx, which in turn, via a series of biochemical processes, leads to the mechanical events. Second, the duration of the action potential is in the order of one to a few milliseconds (longer values with DAP, shorter values in its absence), whereas the mechanical contraction time (at least during isometric contractions) is ~20–25 ms (see Figs. 5 and 6). Thus in most instances the action potential is complete or close enough to being complete before sufficient movement is generated to produce artifacts and/or dislodge the electrode from the fiber. As noted in METHODS, dislodgement of electrodes occurred frequently after one or a few contractions, preventing recording multiple action potentials from a single fiber. However, all that was needed was to record a single action potential from each fiber studied, so this was not a problem. The approach used in the present study has been used previously to record diaphragm action potentials (39,40).

The present study did not measure diaphragm tetanic force, because of concerns of force loss during these measurements possibly affecting subsequent testing of fatigue. In a previous study from our laboratory done by the same person in the same rat gender and strain, we found that normal (sedentary) diaphragm had a twitch-to-tetanic force ratio of 0.24 (38). In the present study, diaphragm twitch forces were 666 and 723 g/cm² for muscle from sedentary and exercised rats, respectively. Assuming that endurance exercise did not alter the twitch-to-tetanic force ratio (a reasonable assumption, because very few other contractile parameters changed, and an alteration of half-relaxation time should not affect tetanic tension), and using the previously determined twitch-to-tetanic force ratio of 0.24, we estimate tetanic forces would have been 2,775 and 3,013 g/cm² for muscle from sedentary and exercised rats, respectively.

In conclusion, voluntary endurance exercise leads to an alteration of diaphragm muscle action potential properties, specifically a slowing of repolarization. The extent of action potential prolongation was not sufficient to increase action potential area, however, which may explain the absent effects on muscle twitch force. The greater prolongation of action potential repolarization by DAP in muscle from sedentary than exercised animals argues that these changes are due to a down-regulation of K+ channels, which could be due to factors such as a diminution of K+ channel opening time, conductance, or density. Further studies are needed to more precisely define the biophysical changes accounting for this phenomenon.

DISCLOSURES

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REFERENCES


