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# Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions

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**McBride, Todd A., Bradley W. Stockert, Fredric A. Gorin, and Richard C. Carlsen.** Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions. *J. Appl. Physiol.* 88: 91–101, 2000.—We tested the hypothesis that eccentric contractions activate mechanosensitive or stretch-activated ion channels (SAC) in skeletal muscles, producing increased cation conductance. Resting membrane potentials and contractile function were measured in rat tibialis anterior muscles after single or multiple exposures to a series of eccentric contractions. Each exposure produced a significant and prolonged (>24 h) membrane depolarization in exercised muscle fibers. The magnitude and duration of the depolarization were related to the number of contractions. Membrane depolarization was due primarily to an increase in $Na^+$ influx, because the estimated $Na^+$-to-$K^+$ permeability ratio was increased in exercised muscles and resting membrane potentials could be partially repolarized by substituting an impermeant cation for extracellular $Na^+$ concentration. Neither the $Na^+/H^+$ antiport inhibitor amiloride nor the fast $Na^+$ channel blocker TTX had a significant effect on the depolarization. In contrast, addition of either of two nonselective SAC inhibitors, streptomycin or $Gd^{3+}$, produced significant membrane repolarization. The results suggest that muscle fibers experience prolonged depolarization after eccentric contractions due, principally, to the activation of $Na^+$-selective SAC.

skeletal muscle; lengthening contraction; resting membrane potential

MULTIPLE EXPOSURES TO RESISTANCE exercise produce adaptive changes in skeletal muscles, including an increase in mass (hypertrophy) and contractile strength (19). Muscle adaptation involves exercise-induced changes in the expression of specific genes and an accompanying increase in protein synthesis (39). A number of cellular pathways have been reported to contribute to the adaptive response, including mechanosensitive cation-selective channels in the muscle membrane (39). Mechanosensitive or stretch-activated ion channels (SAC) may be particularly important when the exercise involves eccentric or lengthening contractions. SAC are a constituent of the cell membrane in most tissues and contribute to the regulation of a wide variety of cellular functions (23, 29). They have been identified in skeletal muscle (13), but their role in muscle function remains uncertain. SAC appear to have an important role in the remodeling of cardiac and smooth muscle cells in response to mechanical strain (17, 18, 30, 31), and they may play a similar role in exercising skeletal muscle.

Several types of SAC have been identified, and one or more of these types appears to be present in most cells (23, 29). In general, SAC provide a major pathway for the transduction of mechanical stimuli in cells, but the subsequent response to the stimulus is tissue specific. In cardiac and smooth muscles, for example, SAC have been linked to mechanically induced cell growth (17, 18), and it appears probable that SAC could contribute to modulating stretch or eccentric exercise-induced cell growth in skeletal muscle as well (37). SAC in cardiac muscle are permeant to monovalent ($K^+$ and $Na^+$) or divalent ($Ca^{2+}$) cations, however, and are associated with membrane depolarization and alterations in action potential generation and conduction (14). Eccentric contraction-induced SAC activation could also contribute to membrane-voltage changes in skeletal muscle, affecting excitation-contraction coupling.

Identifying the physiological role of SAC in any tissue has been hampered by the absence of specific channel inhibitors or activators. Nonetheless, nonselective blockers, including aminoglycoside antibiotics and the trivalent cation gadolinium ($Gd^{3+}$), have been used effectively to inhibit cation-permeable SAC in a variety of tissues, including cardiac and skeletal muscle. Sokabe et al. (33), for example, used patch-clamp methods to show that aminoglycoside antibiotics blocked SAC in cultured chick skeletal muscle cells in a voltage- and dose-dependent manner. Aminoglycosides are viewed as nonselective SAC blockers because they inhibit other types of cation channels as well, but their dissociation constants for SAC are much lower than for the other channels (25). The cation $Gd^{3+}$ is reported to be the most potent SAC blocker available, but it is also nonselective, and its toxicity limits its use to in vitro preparations (14). $Gd^{3+}$ blocks SAC by binding to a site in the permeation pathway for $Na^+$ and $Ca^{2+}$ (23), but it also displaces $Ca^{2+}$ and other ions from specific and nonspecific membrane binding sites, and it can block L-type $Ca^{2+}$ currents (14). Sadoshima et al. (32), however, compared the effect of $Gd^{3+}$ on SAC with its effect on voltage-gated ion channels and concluded that $Gd^{3+}$

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has a higher affinity for SAC than for other cation channels. Application of 10 μM $Gd^{3+}$ to cardiac myocytes, for example, completely inhibited SAC, whereas 50 μM $Gd^{3+}$ produced only a 16% inhibition of voltage-gated $Na^+$ channels and a 32% inhibition of L-type $Ca^{2+}$ channels. Similarly, 10 μM $Gd^{3+}$ produced a significant inhibition (>90%) of stretch-induced arrythmias in isolated perfused dog hearts, primarily as a consequence of its effect on SAC (14).

$Gd^{3+}$ and the aminoglycoside antibiotic streptomycin were used as SAC blockers in the present experiments to help identify conductance pathways that contribute to the significant depolarization observed in muscle cells after eccentric contractile activity. Both agents were applied to isolated muscles maintained in vitro. Rats were also treated with streptomycin before and after exposure to eccentric contractions to inhibit SAC in vivo during the contractile protocol. $Gd^{3+}$ and streptomycin are nonselective channel blockers and may have actions in addition to their effect on SAC, but, they are, at present, the most effective SAC inhibitors available. They were used in conjunction with other $Na^+$ channel blockers as one means of testing the hypothesis that eccentric contractions in skeletal muscle lead to the activation of SAC and an associated increase in $Na^+$ conductance.

## METHODS

Female 3-mo-old Sprague-Dawley rats, with body weights ranging from 240 to 260 g, were used in the experiments. All animal care and use protocols followed approved University of California and National Institutes of Health guidelines. The animals were housed in a temperature-controlled room (19–21°C) with a 12:12-h light-dark cycle. The rats were provided unlimited access to standard rat chow and water. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) within 30 min of engaging in the exercise protocol and before the terminal experiment to evaluate muscle properties. Muscle contractile and electrophysiological properties were evaluated in one group of anesthetized rats immediately (1–2 h) after the exercise protocol. Exercised rats that were to be evaluated at later time points were placed on a warming pad and allowed to recover from the anesthesia. Those animals not immediately evaluated were returned to the animal quarters until the terminal experiment.

*Exercise protocol.* Anesthetized animals performed high- or low-repetition resistance exercise using a pulley device similar to that described by Wong and Booth (40). The rat was placed in the prone position on the supporting platform of the apparatus, which was designed to stabilize the lower leg and allow full ankle rotation. The right hindlimb was passed through a circular opening in the platform, and the knee was stabilized on the platform. The hind foot was attached directly to a plate connected to the lever arm of the pulley system, and the ankle was stabilized with the foot at a 90° angle with respect to the lower leg (neutral position). Two monopolar stainless steel needle electrodes were inserted percutaneously near the sciatic notch to stimulate the sciatic nerve. Supramaximal stimulation of the sciatic nerve above the branch point of the tibial and peroneal nerves caused the plantar flexors (triceps surae) to contract concentrically, stretching the dorsiflexors as they were also maximally activated. The dorsiflexors thus contract eccentrically, in opposition to the stronger ankle extensors. Data reported by Ashton-Miller et al. (3) concerning the biomechanical properties of ankle rotation in the mouse indicate that the dorsiflexors [tibialis anterior (TA) and extensor digitorum longus] and plantar flexors (soleus and plantaris) are at optimum length when the ankle is at the 90° neutral position. They also note that full plantar flexion from the neutral position produces a 10% increase in the length of TA. Supramaximal stimulation consisted of 100-Hz stimulus trains with a train duration of 2.5 s. The low-repetition exercise protocol consisted of four sets of six repetitions (24 repetitions) with a 20-s rest between repetitions and a 5-min rest between sets. Muscles contracted for 1 min over an exercise period of 30 min. The high-repetition protocol consisted of eight segments with four sets of six repetitions in each segment (192 repetitions). Sixty-second rest periods were inserted between the first 16 sets, and 30-s rest periods were inserted between the second 16 sets. A 210-s rest period was inserted between each segment. Muscles contracted for 8 min over an exercise period of 75 min. Exercise protocols were repeated after 10 days (high-repetition group) or 14 days (low-repetition group) in the groups subject to more than one exposure to the protocol.

A separate group of animals was exposed to an exercise protocol consisting only of low-repetition (24 contractions) concentric contractions of the dorsiflexors. The peroneal nerve was surgically exposed and isolated. The exercise device was modified to allow movement in the direction of ankle flexion. The peroneal nerve was placed over a bipolar, platinum-hook electrode and stimulated. The nerve was crushed proximal to the electrode to prevent reflex or antidromic activation of the motor nerves.

*Contractile measurements.* Contractile deficits provide an indirect measure of muscle injury and recovery after eccentric exercise (5, 21). TA contractile function was determined in situ 1, 2, 5, and 14 days after exposure to the low-repetition protocol and 2 and 10 days after exposure to the high-repetition protocol. The animals were anesthetized with pentobarbital sodium (50 mg/kg ip), and the TA and peroneal nerve in each hindlimb were exposed. The rat was placed on a warming pad to maintain body temperature (38 ± 1°C), and the animal and pad were placed on a rat spinal frame. Each hindlimb was immobilized by clamping the knee and ankle joints with clamps fixed to the metal frame. The distal tendon of the TA was isolated and attached to a force transducer (Grass FT-03) with silk suture (4–0). The peroneal nerve was placed on a bipolar platinum-hook electrode and stimulated supramaximally (0.05 ms, 7–10 V). The nerve was laid over the electrode and crushed proximal to the electrode to avoid reflex or antidromic activation of the motor neurons. The TA was adjusted to optimal length by gradually increasing muscle length until maximum twitch amplitude was achieved. Maximum isometric twitch tension, time to peak twitch tension, twitch half relaxation time, rate of twitch relaxation, maximum isometric tetanic tension, and the maximum rate of force development during a tetanus at 330 Hz were recorded at 35 ± 0.5°C.

*Electrophysiology.* Resting membrane potentials (RMPs) were obtained from control and exercised TA both in situ and in vitro. The initial observations in RMP changes in response to eccentric exercise were performed in situ at various time points after either one or two exposures to eccentric contractions. Characterization of muscle membrane conductance after eccentric contractions was performed in vitro, where the composition of the extracellular solution could be controlled and known concentrations of $Na^+$ channel blockers could be applied for a given period and then washed out. The in vitro experiments were used to characterize the effect of $Gd^{3+}$, the most potent SAC inhibitor yet described. Recordings were

obtained by using standard glass microelectrode techniques (4, 26). Electrodes were filled with 3 M KCl and had tip resistances of ~20–30 MΩ. A Grass platinum reference electrode was placed in the proximal end of the TA. RMPs were recorded from TA in situ in rats anesthetized with pentobarbital sodium (50 mg/kg ip). The animals were placed on a thermal pad to maintain body temperature (37 ± 1°C) and were arranged on the rat spinal frame as in the contractile measurements. Control and exercised TA muscles were exposed, cleared of all overlying connective tissue, and covered with warm mineral oil to maintain temperature (35 ± 1°C) and prevent drying. Intracellular recordings were obtained from exercised TA and the contralateral control TA at the end of the exercise protocol and in separate groups of animals at 24, 48, and 72 h postexercise. Each exercise group contained at least four animals, and at least 40 fibers were sampled in each muscle.

RMPs from control and exercised TA were measured in vitro after the muscles were removed from anesthetized animals immediately after the low-repetition exercise protocol. The isolated TA was pinned at rest length to the bottom of a chamber containing 75 ml of HEPES-buffered physiological saline (in mM): 150 NaCl, 5 KCl, 4 $CaCl_2$, 1 $MgCl_2$, 11 glucose, and 1.24 HEPES (6). The saline solution was maintained at room temperature (21 ± 1°C) and continuously bubbled with 100% $O_2$. Fresh solutions were added after the chamber was drained through a vacuum line inserted at the base. In vitro recordings were obtained from muscle cells to a depth of no more than five cells from the surface to avoid recording from the hypoxic core of the muscle. The proximal end of the TA was avoided to prevent recording from fibers damaged by removal of the muscle from the animal. Only one recording was obtained from each fiber.

*Effect of changes in $[K^+]_o$ or substitution for $[Na^+]_o$ on RMP.* The influence of extracellular $[K^+]$ ($[K^+]_o$; brackets denote concentration) on the RMP of control and exercised TA immediately after a first exposure to eccentric exercise was determined in vitro. One leg in the anesthetized rat was exercised in situ. After the exercise, the TA from both the exercised and control legs were rapidly excised and placed in a chamber containing oxygenated HEPES-buffered physiological saline (containing 5 mM $[K^+]$) maintained at room temperature (21°C). $[K^+]$ in the bathing solution was adjusted by reducing $[K^+]_o$ or by substituting $[K^+]_o$ for extracellular $[Na^+]$ ($[Na^+]_o$) on an equimolar basis. Muscle RMP was determined in solutions containing $[K^+]_o$ at concentrations of 0.5, 1.0, 5.0, 10, 50, and 100 mM. The $[K^+][Cl^-]$ product was maintained constant by replacing $[Cl^-]$ with $[SO_4^{2-}]$ when required (16, 34). Membrane potentials were first measured in muscles bathed in the control solution (5 mM $[K^+]_o$). After the control measurements, the 0.5 mM $[K^+]_o$ solution was substituted for the control solution, and RMP was measured. The chamber was drained at the end of each recording period and filled with the solution containing the next highest $[K^+]$. The muscles were allowed to equilibrate in each solution for 10 min before recordings were obtained. RMPs were obtained from at least 20 cells in each muscle at each $[K^+]_o$.

The mean RMPs from control and exercised TA were plotted against $[K^+]_o$. Theoretical relationships between RMP and $[K^+]_o$ were determined by using the Goldman-Hodgkin-Katz equation at a temperature of 21°C

$$\text{RMP} = -58 \text{ mV} \log \frac{[K^+]_i + a[Na^+]_i}{[K^+]_o + a[Na^+]_o}$$

where a is the $Na^+$-to-$K^+$ permeability ratio ($P_{Na^+}/P_{K^+}$).

Values used for $[Na^+]_o$ and $[K^+]_o$ were those of the bathing solution. Values used for intracellular $[Na^+]$ ($[Na^+]_i$) and $[K^+]$ ($[K^+]_i$) were assumed to be 22 and 159 mM, respectively (26). The $P_{Na^+}/P_{K^+}$ was also estimated by using the constant field equation

$$\text{RMP} = \frac{RT}{zF} \ln \frac{[K^+]_o + a[Na^+]_o}{[K^+]_i}$$

where R is the gas constant, $T$ is absolute temperature, z is ion valence, and $F$ is the Faraday constant, rewritten in exponential form. The term $P_{Na^+}[Na^+]_i$ is neglected as being small relative to the terms to which it is added. The graph based on the exponential form of the Goldman-Hodgkin-Katz equation is a straight line, the slope of which is $1/[K^+]_i$ and the $y$-intercept of which is an estimate of the $P_{Na^+}/P_{K^+}$ (1, 10).

In a separate series of experiments, $[Na^+]$ in the bathing solution was replaced by the nonpermeant organic cation *N*-methyl-D-glucamine (NMDG) on an equimolar basis. Muscle RMPs were first recorded in the normal saline solution. After the control measurements, the chamber was drained and rinsed with an oxygenated NMDG solution to remove re-

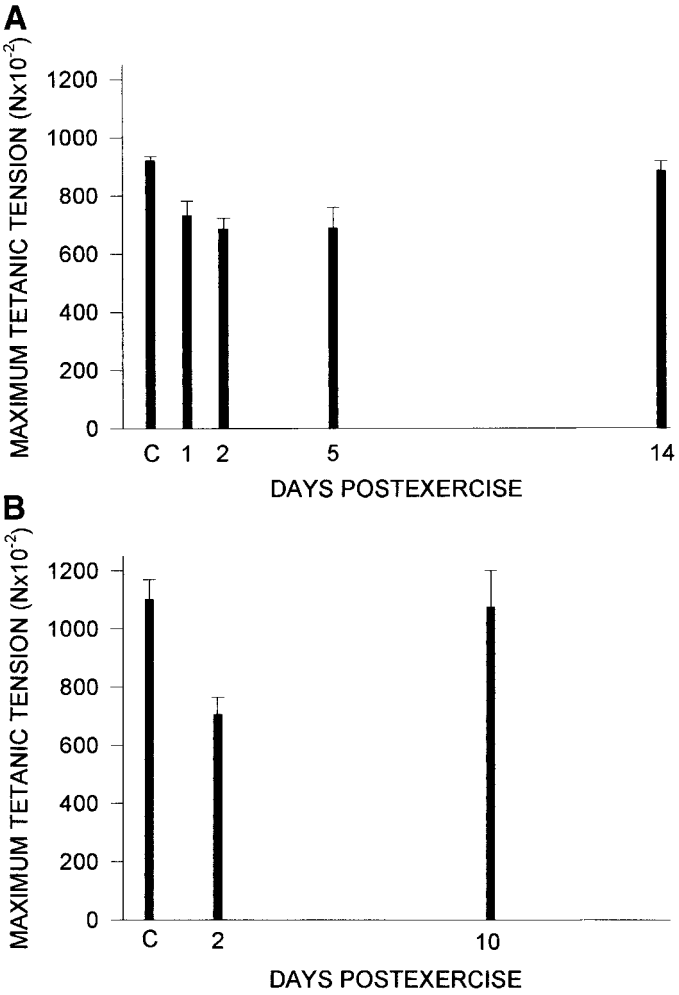


Fig. 1. Effect of initial exposure to low-repetition (*A*; 24 contractions) or high-repetition (*B*; 192 contractions) resistance exercise protocol on maximum tetanic tension that could be generated by exercised tibialis anterior (TA) at indicated no. of days after exercise. Contractile deficit after exposure to eccentric exercise is a reflection of muscle injury produced by exercise (5, 12, 21). C, control TA. Values are means ± SD of measurements from at least 6 animals at each time point (in situ).

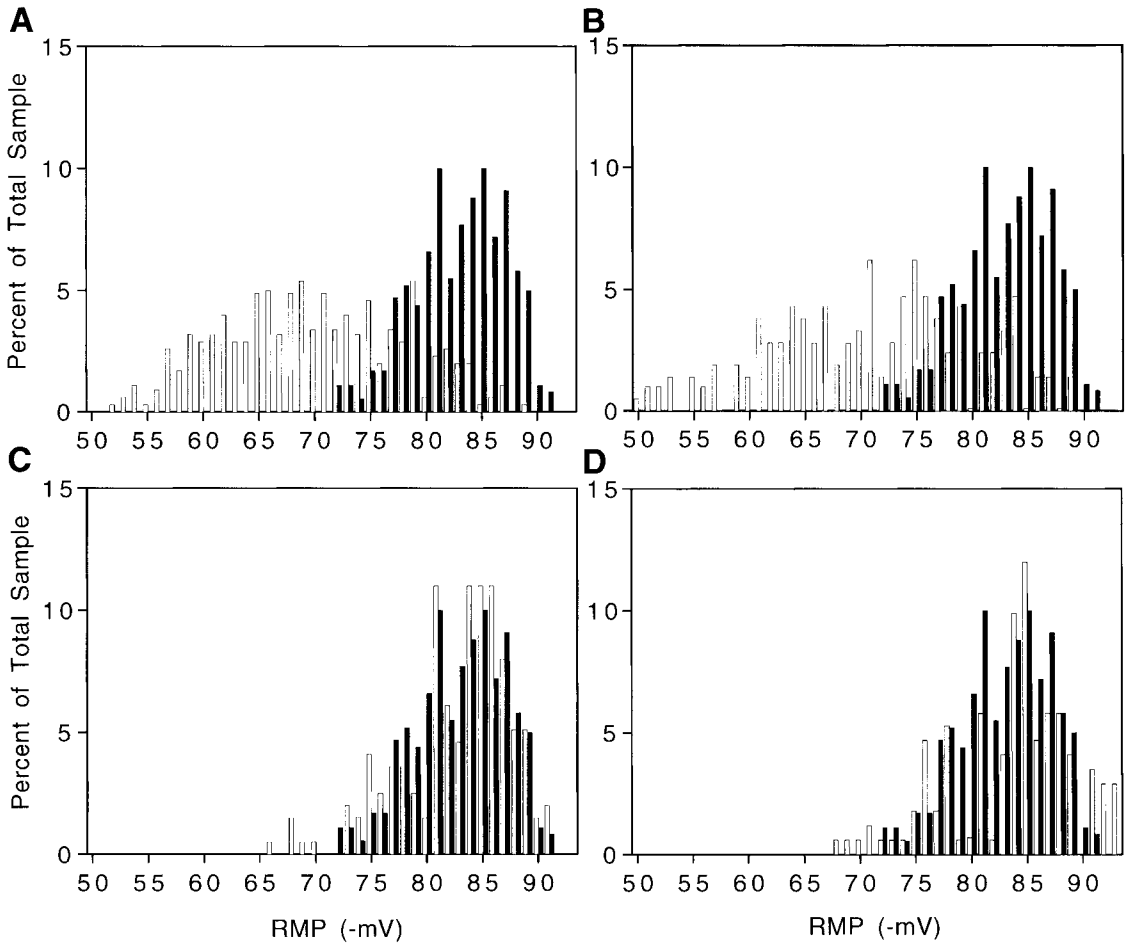


Fig. 2. Frequency distribution of resting membrane potential (RMP) in TA fibers (recorded in situ) after single exposure to low-repetition protocol (solid bars) compared with control fibers in contralateral TA (open bars). *A*: same day after exercise (1–2 h); *B*: 1 day postexercise; *C*: 2 days postexercise; *D*: same day (1–2 h) after low-repetition concentric contractions with use of same protocol as the eccentric contractions. Each set of frequency distributions represents measurements from muscles from at least 4 animals with recordings from at least 40 fibers/muscle.

sidual [$Na^+$] from the chamber. HEPES-buffered physiological solution containing 150 mM NMDG was added to the chamber, and the muscle was allowed to equilibrate for 10 min before recordings were obtained. Recordings were obtained from at least 20 cells in each muscle. Recordings were obtained in three of the five preparations after NMDG was washed out, and the muscle was allowed to equilibrate in normal buffer solution for 10 min.

*Effect of amiloride, TTX, streptomycin, and $Gd^{3+}$ on RMP.* The possible role of specific $Na^+$ channels in the membrane depolarization observed immediately after eccentric exercise was evaluated in vitro. The excised TA was bathed initially in the control oxygenated, HEPES-buffered physiological solution, and a series of RMP measurements was obtained. The chamber was then drained and filled with a solution containing one of the following pharmacological agents. *1*) The $Na^+/H^+$ exchange inhibitor amiloride at concentrations of 0.5 and 1.0 mM was dissolved in DMSO before addition to the bathing solution. The final concentration of DMSO was $<0.05\%$ (vol/vol). Control measurements did not include the addition of DMSO to the bathing solution (11). *2*) The voltage-gated $Na^+$ channel blocker TTX was added at a concentration of $10^{-6}$ M (27). *3*) The SAC blockers streptomycin and $Gd^{3+}$ were added at concentrations of 1 mM and 10 μM, respectively (7, 31). Measurements were obtained from a minimum of 20 fibers/muscle in the presence of each agent.

*Effect of exposure to streptomycin in vivo on the response to eccentric exercise.* Rats were treated with streptomycin in vivo by adding it to the drinking water (4 g/l). Treatment began 6 days before the exercise. RMPs were recorded immediately (1–2 h) after or 1 or 2 days after exposure to eccentric exercise. Isometric contractile properties were evaluated in the low-repetition group 2 days after either the first or second exercise to assess exercise-induced muscle injury in the presence of streptomycin. The TA in the nonexercised, contralateral leg served as the control in each group.

*Statistics.* Results are expressed as means ± SD of the indicated number of measurements. Statistical analyses were performed by using the Statview SE software program (Abacus Concepts, Berkeley, CA). Statistical significance was accepted at $P \leq 0.05$. An ANOVA was used to compare RMP in the exercise groups with the RMP in control groups. Differences indicated by the ANOVA were further analyzed by using the Fisher's *t*-test.

## RESULTS

*Maximum contractile force after initial exposure to eccentric exercise.* A reduction in maximum contractile force has been used as an index of muscle injury after eccentric exercise (5, 21). The initial exposure to low- or high-repetition eccentric exercise was associated with a

significant reduction in maximum isometric tension after the exercise (Fig. 1, *A* and *B*). The low-repetition contractile deficit persisted through 5 days, and force was restored by 14 days postexercise (Fig. 1*A*). High-repetition eccentric exercise produced a force deficit that was greater than the low-repetition deficit (as a percentage of control force) 2 days postexercise (Fig. 1*B*). Maximum force in the exercised TA was not different from the force produced by the contralateral control TA at 10 days postexercise. The maximum rate of tension development and the rate of relaxation of twitch force were also significantly lower 2 days after both the low-repetition (see Table 3) and high-repetition exercise (data not shown). The eccentric exercise protocol used in these experiments produced a significant and reproducible contractile deficit in the exercised TA, as reported previously (20).

*RMP measured in situ after a single exposure to the eccentric exercise protocol.* Muscle cells in the TA showed persistent depolarization after a single exposure to either the low- or high-repetition eccentric exercise protocol. Mean RMP was significantly depolarized within 1–2 h after the exercise (Figs. 2*A* and 3, *A* and *B*) and remained significantly depolarized for 24 h (low repetition, Figs. 2*B* and 3*A*) or 48 h (high repetition, Fig. 3*B*). RMP in exercised muscle fibers returned to control values by 48 h (low repetition, Figs. 2*C* and 3*A*) or 72 h (high repetition, Fig. 3*B*) postexercise. Muscle fibers exposed to the high-repetition protocol were significantly more depolarized than fibers exposed to the low-repetition protocol 1–2 h after the exercise, but depolarization was similar in the two groups at 24 h postexercise. In contrast, the mean RMP of concentrically exercised TA 1–2 h after the exercise was not significantly different from the mean RMP of control TA (Fig. 2*D*; concentrically exercised RMP: $-84.1 \pm 2.5$ mV, $n = 4$ muscles; control RMP: $-83.9 \pm 1.2$ mV; $n = 16$ muscles).

*Influence of $[K^+]_o$ on RMP of control and eccentrically exercised TA.* The low-repetition protocol was used to investigate the possible cellular basis for membrane depolarization after eccentric exercise. Measurements were made on the contralateral control and exercised TA in vitro immediately after the initial exposure to the exercise protocol. The $P_{Na^+}/P_{K^+}$ in muscle cells was evaluated by measuring the response of the RMP to changes in $[K^+]_o$ while holding $[K^+]_o + [Na^+]_o$ constant. The results are shown in Fig. 4. RMPs were recorded from at least 20 fibers in each muscle ($n = 6$). Figure 4*A* shows three theoretical curves calculated from the Goldman constant-field equation for RMP as a function of $[K^+]_o$. The curves assume $P_{Na^+}/P_{K^+}$ values of 0.02, 0.03, and 0.05, respectively. The best-fit relationship between the theoretical curves and the measured values from control TA is provided by the curve where $P_{Na^+}/P_{K^+} = 0.03$. The best-fit relationship for exercised TA is provided by the curve where $P_{Na^+}/P_{K^+} = 0.05$. A linear graph of the data with the use of the exponential form of the Goldman equation (Fig. 4*B*) provided $P_{Na^+}/P_{K^+}$ values of 0.031 for control TA and 0.053 for the eccentrically exercised TA. The difference in $P_{Na^+}/P_{K^+}$ in

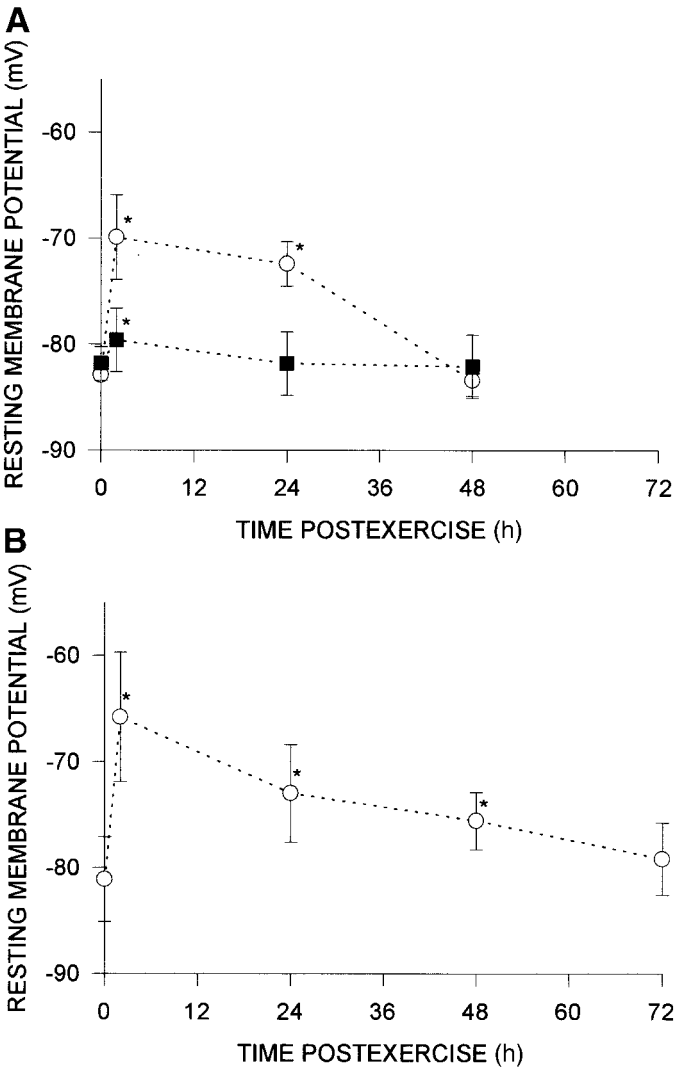


Fig. 3. Means ± SD of RMP recorded from control and exercised TA (in situ) after single exposure to low- (*A*) or high-repetition (*B*) resistance exercise protocol. Each mean value represents measurements from at least 4 animals with recordings from at least 40 fibers/muscle. ■, Recordings from exercised TA pretreated with streptomycin for 6 days before exercise, with treatment continuing until recordings were obtained; ○, recordings from exercised, untreated TA. *Time 0* represents control. Recordings made same day as exercise protocol were taken within 1–2 h after exercise. *Significantly different ($P < 0.05$) from mean RMP of contralateral control TA.

control and exercised TA suggests that the $Na^+$ permeability in exercised muscles increases by 70% within 1–2 h after an initial exposure to the low-repetition protocol.

*Effect of replacement of $[Na^+]_o$ with an impermeant cation on muscle membrane depolarization after eccentric exercise.* The impermeant organic cation NMDG was used to replace $[Na^+]$ in the bathing solution to determine whether $Na^+$ influx contributed to the membrane depolarization observed after eccentric exercise. RMP of fibers in the eccentrically exercised TA, measured in vitro, depolarized from $-81.9 \pm 1.8$ to $-72.7 \pm 0.5$ mV within 1–2 h after an initial exposure to the low-repetition protocol (Table 1). Replacement of $[Na^+]_o$

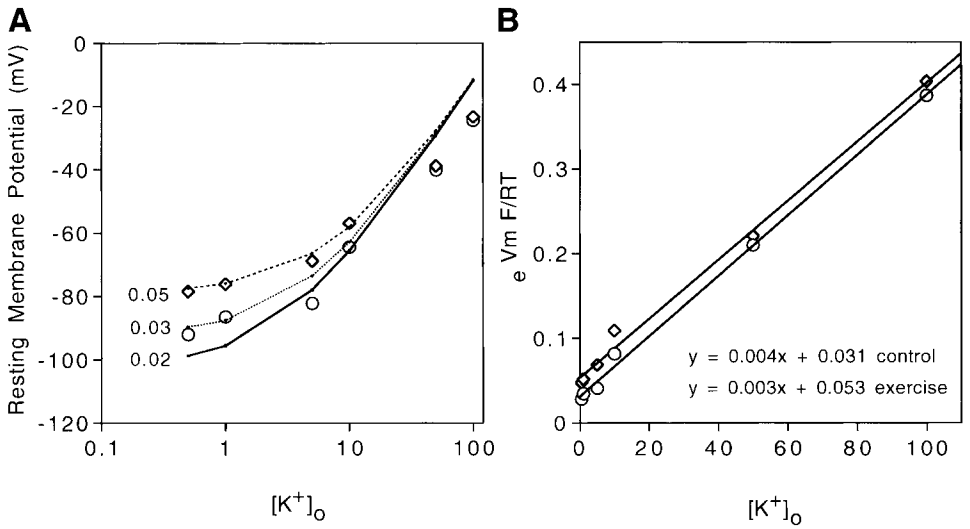


Fig. 4. Mean RMP of muscle fibers (in vitro) exposed to different concentrations of intracellular $K^+$ concentrations ($[K^+]_o$). ○, Mean values for control TA; ◇, mean values for exercised TA 1–2 h after single exposure to low-repetition eccentric exercise protocol. *A*: curves represent calculated values of RMP at various $[K^+]_o$ as determined from Goldman-Hodgkin-Katz equation. Curves are given for permeability ratios of $Na^+$ to $K^+$ ($P_{Na^+}/P_{K^+}$) of 0.02, 0.03, and 0.05 as indicated. Mean values of recorded RMP for exercised and control TA are superimposed on theoretical curves to estimate $P_{Na^+}/P_{K^+}$ for the two groups. Estimated $P_{Na^+}/P_{K^+}$ for control TA was 0.03, and estimated value for exercised TA was 0.05. *B*: mean RMP values from *A* were graphed as an exponential function of the constant field equation. The *y*-intercept is an estimate of $P_{Na^+}/P_{K^+}$ (1, 10). e, Exponential form of equation; Vm, membrane potential; *F*, Faraday constant; *R*, gas constant; *T*, absolute temperature.

with equimolar NMDG produced a repolarization of exercised fibers to −79.1 ± 2.0 mV within 10 min after the change in solutions (Table 1). The NMDG solution was removed and replaced by the control solution, and the exercised muscle cells again depolarized to −71.5 ± 0.7 mV. Control muscles maintained normal RMP in the presence of NMDG (Table 1). These data support the hypothesis that an increase in $Na^+$ permeability, and an accompanying increase in $Na^+$ influx, is primarily responsible for postexercise depolarization of eccentrically exercised muscle cells.

*Which $Na^+$ channels are responsible for the increase in $Na^+$ permeability after eccentric exercise?* Amiloride in high concentrations is reported to block the $Na^+/H^+$ antiporter in many tissues, including skeletal muscle (11). If eccentrically exercised TA depolarized as a consequence of intracellular acidification and a resulting increase in $Na^+/H^+$ exchange, amiloride should block the pathway and produce membrane repolarization. The addition of amiloride to the bathing solution at concentrations of 0.5 and 1.0 mM did not reverse the depolarization observed in exercised muscle cells (Table 1). Addition of amiloride to the bathing solution also had no significant effect on the RMP of control muscles (−82.2 ± 1.2 mV in control fibers compared with −82.5 ± 1.8 mV in 1 mM amiloride). This observation suggests that $Na^+/H^+$ exchange in resting mammalian muscles is less active than in resting frog muscles in which the addition of 0.4 mM amiloride produces a 12-mV hyperpolarization of the muscle (11).

The addition of the voltage-dependent $Na^+$ channel blocker TTX ($10^{-6}$ M) to the bathing solution had no significant effect on the RMP of either control or exercised muscle cells (Table 1). The addition of TTX ($10^{-6}$ M) and amiloride (1 mM) in combination also had

Table 1. *Effect of $Na^+$ substitution and $Na^+$ channel blockers on resting membrane potential of TA (in vitro) subject to low-repetition eccentric exercise*

| | Muscles/ Total Fibers, *n* | Resting Membrane Potential, mV |
|---|---|---|
| Control TA | 22/568 | −81.9 ± 1.8 |
| Control TA (NMDG added) | 4/100 | −82.7 ± 0.7 |
| Exercised TA | 12/360 | −72.7 ± 0.5* |
| Exercised TA (NMDG added) | 5/131 | −79.1 ± 2.0*† |
| Exercised TA (NMDG washout) | 3/70 | −71.5 ± 0.7 |
| Exercised TA (1 mM amiloride added) | 4/111 | −73.5 ± 2.5* |
| Exercised TA ($10^{-6}$ M TTX added) | 2/65 | −73.3 ± 0.6 |

Values are means ± SD of recordings from indicated no. of muscles, with recordings from at least 20 fibers/muscle. TA, tibialis anterior; NMDG, *N*-methyl-D-glucamine. *Significant at $P < 0.05$ compared with control TA; †significant at $P < 0.05$ compared with exercised TA.

Table 2. *Effect of the addition of stretch-activated channel blockers on resting membrane potential of TA (in vitro) subject to low-repetition eccentric exercise*

| | Muscles/ Total Fibers, *n* | Resting Membrane Potential, mV |
|---|---|---|
| Control TA | 22/568 | −81.9 ± 1.8 |
| Exercised TA | 12/360 | −72.7 ± 0.5* |
| Exercised TA (1 mM streptomycin added) | 4/135 | −76.0 ± 1.6*† |
| Exercised TA (10 μM $Gd^{3+}$ added) | 3/105 | −76.7 ± 1.8*† |
| Exercised TA ($Gd^{3+}$ washout) | 2/35 | −73.3 ± 0.7 |

Values are means ± SD of recordings from indicated no. of muscles, with recordings obtained from at least 20 fibers/muscle. *Significant at $P < 0.0001$ compared with control TA; †significant at $P < 0.0001$ compared with exercised TA.

Table 3. *Contractile function in streptomycin-treated and untreated TA 2 days after a single exposure to low-repetition eccentric exercise*

| Group | Muscles, *n* | $P_t$, $N \times 10^{-2}$ | $P_o$, $N \times 10^{-2}$ | $dP_o/dt$, $N \times 10^{-2}$/ms | $dR/dt$, $N \times 10^{-2}$/ms |
|---|---|---|---|---|---|
| Untreated control TA | 6 | 204.0 ± 8.6 | 977.2 ± 39.9 | 42.4 ± 2.1 | 12.7 ± 0.7 |
| Streptomycin-treated control TA | 5 | 188.0 ± 10.9 | 1,010.8 ± 56.4 | 38.8 ± 1.7 | 10.6 ± 1.0 |
| Untreated exercised TA | 6 | 155.8 ± 7.4* | 791.7 ± 45.8* | 34.1 ± 1.7* | 6.9 ± 0.5* |
| Streptomycin-treated exercised TA | 5 | 149.4 ± 16.7† | 862.2 ± 82.5* | 32.2 ± 4.6 | 7.7 ± 1.1† |

Values are means ± SE of measurements from indicated no. of muscles. Streptomycin treatment was started 6 days before the exercise. Measurements were obtained from anesthetized animals in situ. $P_t$, twitch tension; $P_o$, maximum tetanic tension; $dP_o/dt$, maximum rate of tension development during a tetanus at 330 Hz; $dR/dt$, rate of relaxation after a twitch contraction. *Statistically significant at $P < 0.01$ compared with its contralateral control muscle. †Statistically significant at $P < 0.05$ compared with its contralateral control muscle.

no significant effect on depolarized, exercised muscle cells (data not shown). These experiments indicate that the membrane depolarization observed after eccentric muscle contractions is not associated with an increase in $Na^+$ influx through TTX-sensitive $Na^+$ channels or with an increase in $Na^+/H^+$ exchange as a consequence of muscle ischemia (24).

The addition of 1 mM streptomycin [a nonspecific SAC blocker (18)] to the bathing solution led to a significant repolarization of exercised muscle cells (Table 2). Similarly, the addition of $10^{-5}$ M $Gd^{3+}$ (also a nonspecific SAC blocker) to the bathing solution produced a significant repolarization of the exercised muscle (Table 2). Washout of $Gd^{3+}$ in two preparations returned the mean RMP to a value similar to that of untreated, exercised muscles [−73.3 ± 0.7 mV (Table 2)]. Addition of streptomycin and $Gd^{3+}$ in combination produced the same level of repolarization as when each was added separately; i.e., their effects on RMP in exercised TA were not additive (data not shown). This suggests that they are blocking similar conductance channels, most probably SAC, in the muscle fibers.

*Effect of pretreatment with streptomycin in vivo on TA RMP after low-repetition eccentric exercise.* One group of rats received continuous oral administration of streptomycin from 6 days before the first exposure to low-repetition eccentric exercise until the muscle RMP was evaluated at various times after the exercise. Control streptomycin-treated TA had a mean RMP not significantly different from that of control untreated TA (Fig. 3*A*). The mean RMP of exercised TA from the treated group was significantly less depolarized immediately after the first exercise (−79.6 ± 1.3 mV; $n = 5$ muscles) than was the mean RMP of exercised untreated TA (−70.7 ± 1.4 mV; $n = 12$). The streptomycin-treated exercised muscles, however, were still significantly depolarized compared with their controls. The mean RMP of treated exercised TA was not significantly different from that of control, nonexercised TA 1 day after the first exercise, whereas the mean RMP of exercised TA from untreated animals remained significantly depolarized (Fig. 3*A*). Exercised muscles in both treated and untreated groups had a mean RMP similar to that of control TA at 2 days after the exercise.

*Effect of pretreatment with streptomycin in vivo on the TA response to multiple bouts of resistance exercise.* The contractile properties of control, nonexercised muscles were not significantly different between TA treated with streptomycin for 6 days and untreated TA (Tables 3 and 4). The contractile deficit 2 days after the first exposure to the low-repetition exercise was unaffected by treatment with streptomycin (Table 3), despite a significant reduction in the magnitude and duration of the exercise-induced membrane depolarization produced by streptomycin treatment (Fig. 3*A*). A second exposure to the low-repetition protocol, 14 days after the first, did not produce a significant contractile deficit 2 days after the exercise (Table 4). In contrast, TA muscles exposed to a second low-repetition exercise were still significantly depolarized immediately after the exercise (Figs. 5*A* and 6). Pretreatment with streptomycin for 6 days before the first exercise increased the magnitude and duration of the exercise-induced depolarization after the second exercise (Figs. 5*B* and 6). The membrane voltage changes in the pretreated TA after the second exercise resembled the changes seen in the untreated TA after the first exposure to the exercise (Fig. 2, *A* and *B*).

Exposure to multiple bouts of the high-repetition protocol was associated with muscle adaptation, seen as a decrease in the contractile deficit measured 2 days after the exercise. Maximum tetanic force was 82 ±

Table 4. *Contractile function in streptomycin-treated and untreated TA 2 days after a second exposure to low-repetition eccentric exercise*

| Group | Muscles, *n* | $P_t$, $N \times 10^{-2}$ | $P_o$, $N \times 10^{-2}$ | $dP_o/dt$, $N \times 10^{-2}$/ms | $dR/dt$, $N \times 10^{-2}$/ms |
|---|---|---|---|---|---|
| Untreated control TA | 5 | 211.0 ± 6.7 | 1,147.4 ± 34.1 | 48.9 ± 1.7 | 10.6 ± 0.9 |
| Untreated exercised TA | 5 | 217.8 ± 13.9 | 1,107.0 ± 33.6 | 44.4 ± 2.3 | 9.2 ± 0.9 |
| Streptomycin-treated control TA | 7 | 200.3 ± 11.0 | 1,004.6 ± 15.0 | 38.4 ± 1.4 | 11.3 ± 1.0 |
| Streptomycin-treated exercised TA | 7 | 187.1 ± 9.7 | 953.7 ± 43.2 | 36.6 ± 2.4 | 9.1 ± 1.3 |

Values are means ± SE of measurements from indicated no. of muscles. Streptomycin was started 6 days before first exercise in all of treated animals. Streptomycin was discontinued at the time of the first exercise.

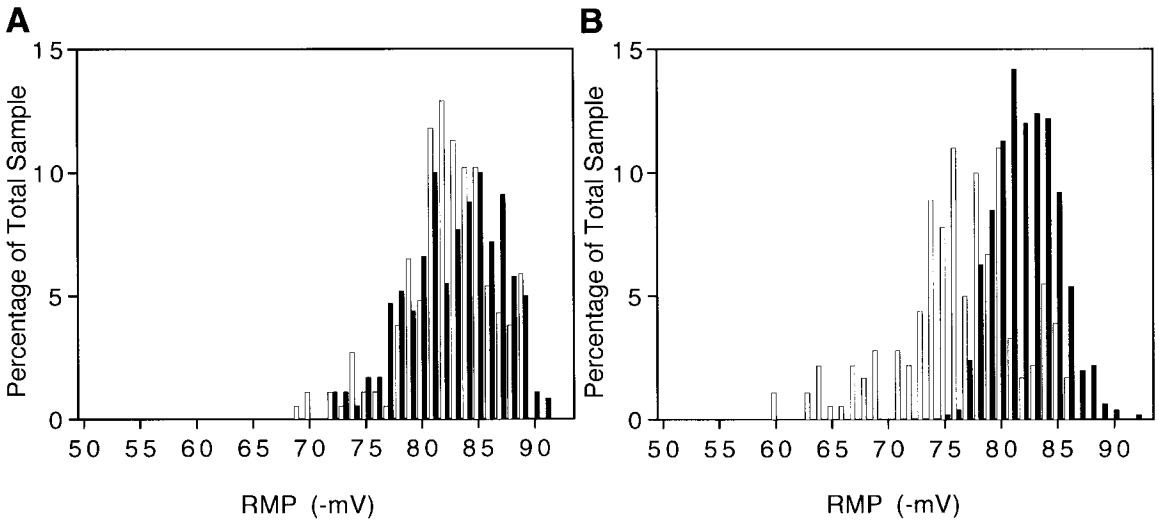


Fig. 5. Frequency distribution of RMP in TA (recorded in situ) 1 day after second exposure to low-repetition protocol. One group of rats was treated with streptomycin starting 6 days before first exercise. Treatment was discontinued between first and second exposure to contraction protocol. *A*: distribution of RMP in exercised (open bars) and contralateral control (solid bars) TA from untreated animals 1 day after exercise. There was no significant difference between the 2 populations. *B*: distribution of RMP in exercised and contralateral control TA from streptomycin-treated animals 1 day after exercise. Mean RMP of treated, exercised fibers (−76.2 ± 0.7 mV) was significantly different ($P < 0.05$) from mean RMP of treated control fibers (−82.7 ± 0.4 mV). Each set of frequency distributions represents measurements from at least 4 animals with recordings from at least 40 fibers/muscle.

13% ($n = 7$) of contralateral control force 2 days after the second exposure to the protocol and 89 ± 7% ($n = 18$) of control 2 days after the third exposure. The first exposure to the high-repetition protocol, in contrast, produced a maximum tetanic force that was 64 ± 8% ($n = 16$) of control 2 days after the exercise (Fig. 1*B*). The magnitude of membrane depolarization was less after the second and third exercise than after the first exercise (Figs. 3*B* and 7), but RMP was still significantly depolarized immediately and at 24 h after both the second and third exercise (Fig. 7). The mean RMP of exercised TA was not significantly different from the mean control value at 48 h after the second and third exercise. Pretreatment with streptomycin starting 6 days before the final exercise reduced (second exercise) or prevented (third exercise) RMP depolarization after the exercise (Fig. 7).

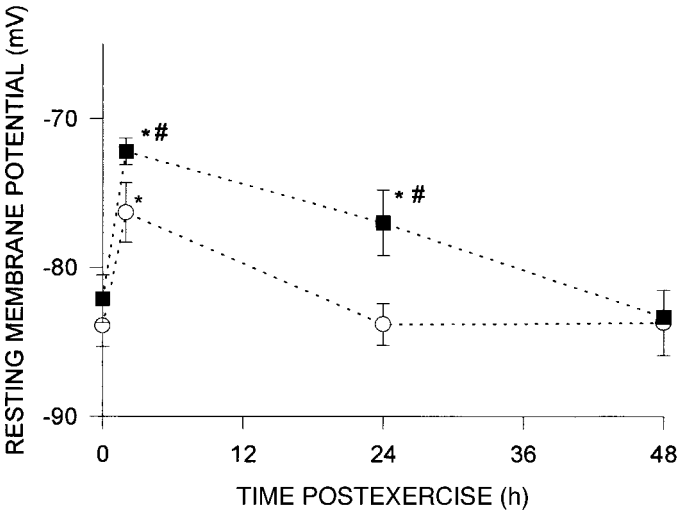


Fig. 6. Means ± SD of RMP (in situ) recorded from control and exercised TA after second exposure to low-repetition resistance exercise protocol 14 days after first exposure. Each mean value represents measurements from at least 4 animals with recordings from at least 40 fibers/muscle. ■, Recordings from exercised TA pretreated with streptomycin for 6 days before and continuing through the first exercise; ○, recordings from untreated, exercised TA. Treatment was discontinued after the first exercise. *Time 0* represents control. Recordings made same day as exercise protocol were taken within 1–2 h after exercise. Significant difference ($P < 0.05$) from *control TA, and #untreated, exercised TA.

## DISCUSSION

Eccentric contractions in the predominately fast-twitch rat TA induced a significant, prolonged depolarization of the muscle. The magnitude and duration of the depolarization were related to the number of contractions during resistance exercise and to the number of previous exposures to the exercise protocol. Depolarization was greatest and most prolonged after the first exposure to the exercise, but subsequent exposures also produced significant, prolonged depolarization. Exposure to the same number of concentric contractions did not produce a significant change in muscle RMP. In vitro measurements showed that the membrane depolarization was due, for the most part, to an increase in $Na^+$ influx triggered by the eccentric contractions. Experiments comparing the ability of specific $Na^+$ blockers to reverse the postexercise depolarization showed that the $Na^+/H^+$ antiport inhibitor amiloride and the voltage-gated $Na^+$ channel inhibitor TTX did not affect the depolarization. In contrast, the addition of either of two nonspecific SAC inhibitors, streptomycin or $Gd^{3+}$, led to a significant repolarization of the membrane. The two agents are not selective for specific mechanosensitive ion channels, but they have been

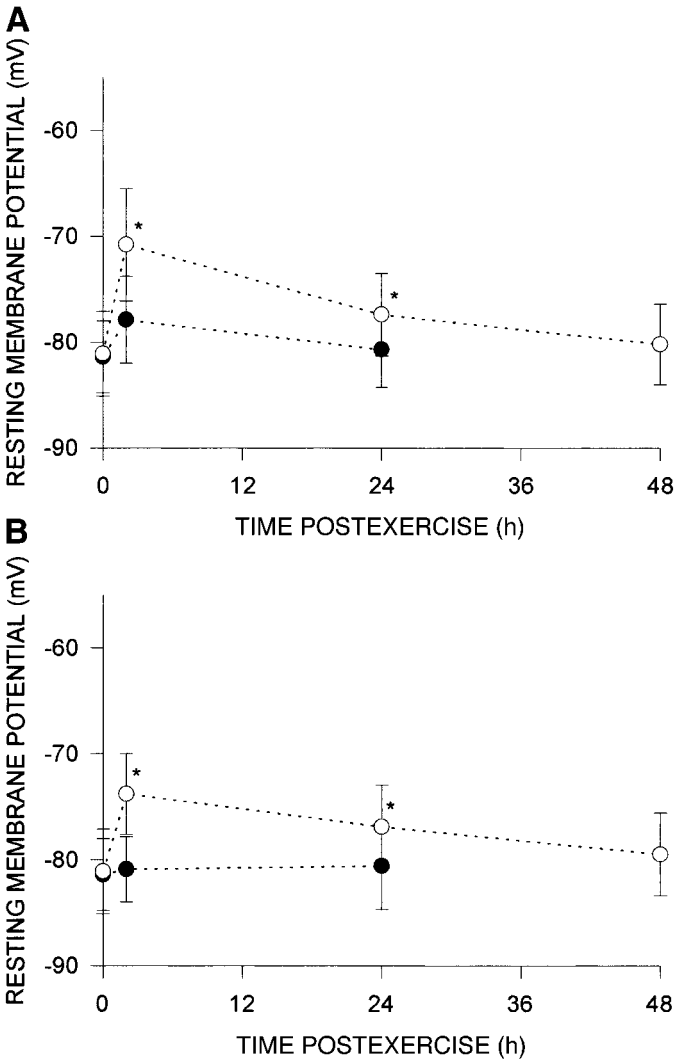


Fig. 7. RMP means ± SD (in situ) of control and exercised TA at indicated times after second (*A*) and third (*B*) exposure to high-repetition protocol. Each exercise exposure occurred 10 days after previous exposure. ○, Measurements from untreated, exercised TA; ●, measurements from TA in rats treated with streptomycin beginning 6 days before indicated exercise and continuing until recordings were obtained. *Time 0* indicates mean of control measurements. Recordings indicated as made same day of exercise were made 1–2 h post-exercise. Values are means of RMP from at least 4 animals per time point, with recordings obtained from at least 40 fibers per muscle. *Significantly different ($P < 0.05$) from control and treated TA.

shown to inhibit ion conductance through SAC in several different systems (29, 33). The effect of the two agents given together at supramaximal concentrations was not additive, which also suggests that they were acting on the same conductance pathway. The results are consistent with a prolonged, exercise-induced activation of mechanosensitive cation channels (SAC) in eccentrically contracting skeletal muscle.

The activation of SAC produced by eccentric contractions could contribute to the muscle response to eccentric exercise in several ways. $Na^+$ and/or $Ca^{2+}$ influx through SAC may initiate a cascade of events leading to the delayed, or secondary, contraction-induced injury and reduction in contractile capacity observed several days after eccentric exercise (12). Increases in $[Na^+]_i$ and intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$), for example, are associated with the contractile and metabolic deficits observed after prolonged ischemia and reperfusion of skeletal muscle (38). It has been difficult, however, to demonstrate a clear association between an increase in $[Ca^{2+}]_i$ and contraction-induced muscle injury (2). Moreover, the present experiments suggest that SAC-associated ion currents were not associated with postexercise muscle deficits. Treatment of the rats with streptomycin in vivo to block SAC before and during the first exposure to the low-repetition protocol prevented membrane depolarization after the eccentric contractions but did not prevent the reduction in maximum isometric contractile force observed 2 days after the exercise (Table 3). Thus SAC-associated ion currents do not appear to contribute to muscle injury or force deficits after eccentric contractions.

Ionic permeability increased after the first exposure to eccentric exercise, but the increase was not due entirely to the activation of SAC. Treatment with streptomycin before the first low-repetition or the second high-repetition exercise did not prevent a significant depolarization 1–2 h after the exercise, suggesting that pathways other than SAC also contributed to the increase in membrane permeability. The other pathways have yet to be identified, although $Na^+/H^+$ exchange and voltage-dependent fast $Na^+$ channels may presumably be ruled out, because neither amiloride nor TTX had any significant effect on the postexercise depolarization. Eccentric exercise is associated with sarcolemmal injury, which is most prominent after the first exposure to the exercise (8, 22). Sarcolemmal injury is characterized by the leak of large molecules, including creatine kinase and lactic dehydrogenase, out of the muscle (2, 8) and the movement of serum albumin into the muscle (22). Membrane damage could also produce nonspecific increases in membrane permeability to all small ions, including $Na^+$ and $Ca^{2+}$, and contribute to membrane depolarization, contractile deficits, and muscle injury. We speculate that the initial depolarization measured after a novel exposure to an eccentric exercise protocol is due to a combination of both membrane damage and the opening of SAC.

Nonspecific permeability changes produced by membrane damage are clearly reduced with continuing exposure to a resistance exercise protocol (8), but SAC activation appears to accompany each exposure to the protocol. Addition of streptomycin before the third exposure to the high-repetition protocol completely blocked the postexercise membrane depolarization (Fig. 7), suggesting that depolarization after this exercise was due entirely to SAC activation. The SAC-induced depolarization was also prolonged, indicating that the increase in $Na^+$ influx was maintained well past the period of active contraction. In cardiac muscle, SAC are directly gated by mechanical stimuli and are slow to desensitize or adapt (14). SAC identified in cardiac myocytes, for example, can maintain their open state for long periods of stretch without adaptation (28). The prolonged SAC-mediated depolarization in eccentri-

cally exercised TA suggests that mechanical strain at the molecular level may persist well beyond the period of exercise, possibly as a consequence of cellular edema or cytoskeletal changes related to the lengthening contractions. Sustained SAC activation could mediate the long-term changes in muscle associated with repeated exposure to resistance exercise (19).

SAC contribute to a wide array of cellular activities, including volume regulation, electrolyte homeostasis, sensory transduction, and cell growth (29). SAC in cardiac and smooth muscle cells modulate electrical and mechanical activity (14, 31, 36, 41) and appear to contribute to the cellular hypertrophy produced by volume overload or mechanical stretch (17, 18, 31). The role of SAC in skeletal muscle has yet to be determined, but there are considerable similarities in the response of cardiac and skeletal muscle cells to sustained mechanical stretch. Stretch stimulates an increase in gene expression, an increase in protein synthesis, and the development of cellular hypertrophy in both types of muscle (15, 17, 31, 39). Sudden or sustained stretch in both muscle types also leads to membrane depolarization linked to the activation of SAC (41). $Na^+$ influx through SAC is reported to stimulate an increase in protein synthesis and cell growth in the mammalian myocardium (17), although this relationship has not been established in skeletal muscle. Growth in both cardiac and skeletal muscles has also been linked to activation of the $Na^+$ pump (17, 36), which could be driven by a SAC-mediated increase in $Na^+$ influx. The magnitude and duration of a change in pump activity, however, will vary, depending on the magnitude and duration of the increase in $Na^+$ influx. The muscle may adapt to continuing stimulation by increasing the number of pump sites in the membrane (9), which might affect the contribution of either increased $Na^+$ influx or increased $Na^+$ pump activity to muscle growth.

*Summary.* The experiments reported in the present study were designed to test the hypothesis that the mechanical strain produced by lengthening or eccentric contractions in skeletal muscle fibers activates SAC in the fibers. The experiments characterized the SAC contribution to the magnitude and duration of changes in RMP after one or more exposures to low- or high-repetition eccentric contractions. The results indicated that skeletal muscle cells were significantly depolarized soon after (1–2 h) and remained significantly depolarized for at least 24 h after exposure to even low-repetition contractions. The magnitude and duration of the postexercise depolarization, however, were related to the intensity (number of eccentric contractions) and to the number of previous exposures to the exercise. The results also suggest that postexercise depolarization occurred as a consequence of an increase in $Na^+$ permeability in the muscle. The increase in permeability observed after the initial exposure to a series of eccentric contractions may involve both a nonspecific increase associated with damage to the sarcolemma and an increase due to the activation of SAC. Repeated exposures appear to activate only the SAC pathway. SAC, however, did not appear to contribute to the force deficit after the first exposure, because blocking SAC with streptomycin did not reduce the deficit. Nonetheless, SAC activation in skeletal muscle may contribute to the stimulation of specific gene expression and protein synthesis (35), as is the case in cardiac and smooth muscle cells exposed to prolonged mechanical strain (17, 18, 31). We speculate that SAC activation may serve as an initial step in muscle adaptation and in the development of muscle hypertrophy during training involving eccentric contractions.

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## REFERENCES

1. **Akaike, N.** Contribution of an electrogenic sodium pump to membrane potential in mammalian skeletal muscle fibres. *J. Physiol. (Lond.)* 245: 499–520, 1975.
2. **Armstrong, R. B.** Mechanisms of exercised-induced delayed onset muscular soreness: a brief review. *Med. Sci. Sports Exerc.* 16: 529–538, 1984.
3. **Ashton-Miller, J. A., Y. He, V. A. Kadhiresan, D. A. McCubbrey, and J. A. Faulkner.** An apparatus to measure in vivo biomechanical behavior of dorsi- and plantarflexors of mouse ankle. *J. Appl. Physiol.* 72: 1205–1211, 1992.
4. **Atrakchi, A., S. D. Gray, and R. C. Carlsen.** Development of soleus muscles in SHR: relationship of muscle deficits to rise in blood pressure. *Am. J. Physiol. Cell Physiol.* 267: C827–C835, 1994.
5. **Brooks, S. V., E. Zerba, and J. A. Faulkner.** Injury to muscle fibres after single stretches of passive and maximally stimulated muscle in mice. *J. Physiol. (Lond.)* 488: 459–469, 1995.
6. **Brown, M. C., and R. Ironton.** Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. *J. Physiol. (Lond.)* 278: 325–348, 1978.
7. **Bustamante, J. O., A. Ruknudin, and F. Sachs.** Stretch-activated channels in heart cells: relevance to cardiac hypertrophy. *J. Cardiovasc. Pharmacol.* 17, *Suppl.* 2: S110–S113, 1991.
8. **Clarkson, P. M., and I. Tremblay.** Exercise-induced muscle damage, repair, and adaptation in humans. *J. Appl. Physiol.* 65: 1–6, 1988.
9. **Clausen, T., and O. B. Nielsen.** The $Na^+$, $K^+$-pump and muscle contractility. *Acta Physiol. Scand.* 152: 365–373, 1994.
10. **Delbono, O., and B. A. Kotsias.** Hyperpolarizing effect of aminophylline, theophylline, and cAMP on rat diaphragm fibers. *J. Appl. Physiol.* 64: 1893–1899, 1988.
11. **Estrada, F., and J. A. Sanchez.** The effect of amiloride on the resting membrane potential and the electrical constants of frog skeletal muscle fibres. *J. Physiol. (Lond.)* 433: 705–717, 1991.
12. **Faulkner, J. A., S. V. Brooks, and E. Zerba.** Muscle atrophy and weakness with aging: contraction-induced injury as an underlying mechanism. *J. Gerontol. A Biol. Sci. Med. Sci.* 50A: 124–129, 1995.
13. **Franco, A., Jr., and J. B. Lansman.** Stretch-sensitive channels in developing muscle cells from a mouse cell line. *J. Physiol. (Lond.)* 427: 361–380, 1990.
14. **Franz, M. R.** Mechano-electrical feedback in ventricular myocardium. *Cardiovasc. Res.* 32: 15–24, 1996.
15. **Goldspink, G., A. Scutt, P. T. Loughna, D. J. Wells, T. Jaenicke, and G. F. Gerlach.** Gene expression in skeletal muscle in response to stretch and force generation. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 262: R356–R363, 1992.
16. **Hodgkin, A. L., and P. Horowicz.** The influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol. (Lond.)* 148: 127–160, 1959.

17. **Kent, R. L., J. K. Hoober, and G. Cooper.** Load responsiveness of protein synthesis in adult mammalian myocardium: role of cardiac deformation linked to sodium influx. *Circ. Res.* 64: 74–85, 1989.
18. **Komuro, I., S. Kudo, T. Yamazaki, Y. Zou, I. Shiojima, and Y. Yazaki.** Mechanical stretch activates the stress-activated protein kinases in cardiac myocytes. *FASEB J.* 10: 631–636, 1996.
19. **Kraemer, W. J., S. J. Fleck, and W. J. Evans.** Strength and power training: physiological mechanisms of adaptation. *Exerc. Sport Sci. Rev.* 24: 363–397, 1996.
20. **McBride, T. A., F. A. Gorin, and R. C. Carlsen.** Prolonged recovery and reduced adaptation in aged rat muscle following eccentric exercise. *Mech. Ageing Dev.* 83: 185–200, 1995.
21. **McCully, K. K., and J. A. Faulkner.** Injury to skeletal muscle fibers of mice following lengthening contractions. *J. Appl. Physiol.* 59: 119–126, 1985.
22. **McNeil, P. L., and R. Khakee.** Disruptions of muscle fiber plasma membranes. *Am. J. Pathol.* 140: 1097–1109, 1992.
23. **Morris, C. E.** Mechanosensitive ion channels. *J. Membr. Biol.* 112: 93–107, 1990.
24. **Murphy, E., M. Perlman, R. London, and C. Steenbergen.** Amiloride delays the ischemia-induced rise in cytosolic free calcium. *Circ. Res.* 68: 1250–1258, 1991.
25. **Nomura, K., K. Naruse, K. Watanabe, and M. Sokabe.** Aminoglycoside blockade of $Ca^{2+}$ activated $K^+$ channel from rat brain synaptosomal membranes incorporated into planar bilayers. *J. Membr. Biol.* 115: 241–251, 1990.
26. **Pickar, J. G., R. C. Carlsen, A. Atrakchi, and S. D. Gray.** Increased $Na^+$-$K^+$ pump number and decreased activity of soleus muscles in SHR. *Am. J. Physiol. Cell Physiol.* 267: C836–C844, 1994.
27. **Pickar, J. G., S. J. Spier, J. R. Snyder, and R. C. Carlsen.** Altered ionic permeability in skeletal muscle from horses with hyperkalemic periodic paralysis. *Am. J. Physiol. Cell Physiol.* 260: C926–C933, 1991.
28. **Ruknudin, A., F. Sachs, and J. O. Bustamante.** Stretch-activated ion channels in tissue-cultured chick heart. *Am. J. Physiol. Heart Circ. Physiol.* 264: H960–H972, 1993.
29. **Sackin, H.** Mechanosensitive channels. *Annu. Rev. Physiol.* 57: 333–353, 1995.
30. **Sadoshima, J., and I. Izumo.** Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. *EMBO J.* 12: 1681–1692, 1993.
31. **Sadoshima, J., and I. Izumo.** Mechanotransduction in stretch-induced hypertrophy of cardiac myocytes. *J. Recept. Res.* 13: 777–794, 1993.
32. **Sadoshima, J., T. Takahashi, L. Jahn, and S. Izumo.** Roles of mechano-sensitive ion channels, cytoskeleton and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. *Proc. Natl. Acad. Sci. USA* 89: 9905–9909, 1992.
33. **Sokabe, M., N. Hasegawa, and K. Yamamori.** Blockers and activators for stretch-activated ion channels of chick skeletal muscle. *Ann. NY Acad. Sci.* 707: 417–420, 1993.
34. **Sperelakis, N., and K. Shigenobu.** Changes in membrane properties of chick embryonic hearts during development. *J. Gen. Physiol.* 60: 430–453, 1972.
35. **Stockert, B., and R. Carlsen.** The effect of eccentric exercise on cellular and molecular characteristics of rat tibialis anterior (Abstract). *Physiologist* 39: A-75, 1996.
36. **Vandenburgh, H. H.** Relationship of muscle growth in vitro to sodium pump activity and transmembrane potential. *J. Cell. Physiol.* 119: 283–295, 1984.
37. **Vandenburgh, H. H.** Mechanical forces and their second messengers in stimulating cell growth in vitro. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 262: R350–R355, 1992.
38. **Welsh, D. G., and M. I. Lindinger.** L-type $Ca^{2+}$ channel and $Na^+/Ca^{2+}$ exchange inhibitors reduce $Ca^{2+}$ accumulation in reperfused skeletal muscle. *J. Appl. Physiol.* 80: 1263–1269, 1996.
39. **Williams, R. S., and P. D. Neufer.** Regulation of gene expression in skeletal muscle by contractile activity. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, chapt. 25, p. 1124–1150.
40. **Wong, T. S., and F. W. Booth.** Skeletal muscle enlargement with weight-lifting exercise by rats. *J. Appl. Physiol.* 65: 950–954, 1988.
41. **Zabel, M., B. S. Koller, F. Sachs, and M. R. Franz.** Stretch-induced voltage changes in the isolated beating heart: importance of the timing of stretch and implications for stretch-activated ion channels. *Cardiovasc. Res.* 32: 120–130, 1996.