K<sub>ATP</sub> channels depress force by reducing action potential amplitude in mouse EDL and soleus muscle

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Gong, B., D. Legault, T. Miki, S. Seino, and J. M. Renaud. K<sub>ATP</sub> channels depress force by reducing action potential amplitude in mouse EDL and soleus muscle. Am J Physiol Cell Physiol 285: C1464–C1474, 2003. First published August 13, 2003; 10.1152/ajpcell.00278.2003.—Although ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channel openers depress force, channel blockers have no effect. Furthermore, the effects of channel openers on single action potentials are quite small. These facts raise questions as to whether 1) channel openers reduce force via an activation of K<sub>ATP</sub> channels or via some nonspecific effects and 2) the reduction in force by K<sub>ATP</sub> channels operates by changes in amplitude and duration of the action potential. To answer the first question we tested the hypothesis that pinacidil, a channel opener, does not affect force during fatigue in muscles of Kir6.2<sup>−/−</sup> mice that have no cell membrane K<sub>ATP</sub> channel activity. When wild-type extensor digitorum longus (EDL) and soleus muscles were stimulated to fatigue with one tetanus per second, pinacidil increased the rate at which force decreased, the reduction in action potential amplitude and if abolishing K<sub>ATP</sub> channel activity, either by blocking the channels or disrupting the Kir6.2 gene, has little effect on force because of lower myosin ATPase activity because of fewer actomyosin links.

With the use of channel openers, several studies have indeed given evidence that the activation of K<sub>ATP</sub> channels causes faster decreases in Ca<sup>2+</sup> release and force during fatigue (9, 16), anoxia (30), and metabolic inhibition (5). However, despite the evidence for the activation of K<sub>ATP</sub> channels under control conditions (i.e., no drug), most studies fail to show an effect of blocking K<sub>ATP</sub> channel on Ca<sup>2+</sup> release and force during fatigue, anoxia, and metabolic inhibition in amphibian (14) and mammalian (9, 16, 28, 30) muscle. Even muscles that have no K<sub>ATP</sub> channel activity in cell membrane (i.e., those from a mouse null for the Kir6.2 gene; Ref. 17) have a similar rate of fatigue compared with muscles from wild-type mice (11).

Furthermore, modulating K<sub>ATP</sub> channel activity has a small effect on single action potentials. In nonfatigued mouse extensor digitorum longus (EDL) and soleus muscle, pinacidil, a K<sub>ATP</sub> channel opener, reduces action potential overshoot by <8 mV (16). Such a decrease in overshoot is too small to reduce twitch force (32). Although K<sub>ATP</sub> channels prevent large prolongation of action potential duration during fatigue in amphibian muscle, the action potential duration measured after fatigue is still longer than before fatigue (14). Glibenclamide reduces the duration of single action potentials during metabolic inhibition in frog muscles (12), but the effect is again quite small.

If K<sub>ATP</sub> channels have small effects on single action potential amplitude and if abolishing K<sub>ATP</sub> channel activity, either by blocking the channels or disrupting the Kir6.2 gene, has little effect on force, this raises questions as to 1) whether channel openers reduce force via an activation of K<sub>ATP</sub> channels or via some nonspecific effects and 2) whether the reduction of force...
by K<sub>ATP</sub> channels operates by changes in the amplitude and duration of action potential.

An answer to the first question is important because it is still uncertain as to whether K<sub>ATP</sub> channels suppress force. Therefore, to answer the first question we tested the hypothesis that if pinacidil depresses force in wild-type muscles solely via the activation of K<sub>ATP</sub> channels, pinacidil will have no effect in Kir6.2<sup>−/−</sup> muscles. The results show that pinacidil does not affect action potentials and the kinetics of fatigue in EDL and soleus muscles of Kir6.2<sup>−/−</sup> mice.

It then became important to answer the second question because so far there is no evidence that the depression of action potentials by K<sub>ATP</sub> channels is large enough to affect force. However, although the effects of K<sub>ATP</sub> channels on force have been determined on tetanic contractions, the effects on membrane excitability have been studied only on single action potentials (12, 14, 16). Therefore, to answer the second question we tested the hypothesis that K<sub>ATP</sub> channels have greater effects on membrane excitability during action potential trains, especially during metabolic stress such as fatigue. The results show that K<sub>ATP</sub> channels significantly reduce the capacity of cell membrane to generate action potential train in wild-type muscles during fatigue.

**METHODS AND MATERIALS**

**Animals and Muscle Preparation**

Kir6.2<sup>−/−</sup> mice were generated as previously described by Miki et al. (18). Kir6.2<sup>−/−</sup> mice did not require any special care. C57BL6 mice were used as wild-type mice. Mice were bred, fed ad libitum, and housed according to the guidelines of the Canadian Council for Animal Care. The Animal Care Committee of the University of Ottawa approved all experimental procedures. Mice were injected intraperitoneally with pentobarbital sodium at a dose of 0.8 mg/10 g body wt for anesthesia before dissection of EDL and soleus muscles.

**Solutions**

Muscles were constantly immersed in physiological saline solution. The control solution contained (in mM) 118.5 NaCl, 4.7 KCl, 2.4 CaCl<sub>2</sub>, 3.1 MgCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 2 NaH<sub>2</sub>PO<sub>4</sub>, and 5.5 D-glucose. All solutions were continuously bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> and had a pH of 7.4. Pinacidil-containing solutions were prepared by first dissolving pinacidil in DMSO, which was then added to the control solution. The pinacidil concentration was 100 µM, and the DMSO concentration was 0.1% in all solutions (including the control solution). Pinacidil activates K<sub>ATP</sub> channels in intact flexor digitorum muscle fibers, with a maximum effect at 100 µM (4). Experiments were carried out at 37°C, except for action potential measurements, which were obtained at 33°C because lower temperatures gave better cell membrane stability.

**Stimulation**

Tetanic contractions were elicited with a 200-ms train of 0.3-ms, 10-V (supramaximal voltage) pulses at 140 Hz for soleus muscle and 200 Hz for EDL muscle. The stimulating currents, which passed between parallel platinum wires located on opposite sides of the muscle, were generated with a Grass S88 stimulator and a Grass SIU5 isolation unit. Grass, West Warwick, RI). All muscles were allowed 30 min to equilibrate in the absence or presence of 100 µM pinacidil before any measurement. During that time one tetanic contraction was elicited every 2 min. Fatigue was elicited with one tetanic contraction every second for 3 min. After fatigue, muscles were stimulated at 10, 20, 100, and 200 s and 5 min and every 5 min thereafter to measure force recovery.

**Force Measurements**

Force measurement was as described by Matar et al. (16). Briefly, paired muscles from each mouse were used simultaneously; one muscle was used as control and the other was exposed to 100 µM pinacidil. Muscle tendons were tied with surgical silk (6-0) to allow attachment of muscle to the experimental apparatus. Muscle length was adjusted to obtain maximum tetanic force. Force was measured with a Kulite semiconductor strain gauge (model BG100, Vancouver, BC, Canada) and digitized at 5 kHz with a Keithley Metrabyte analog-digital board (model DAS50, Edmonton, AB, Canada). Peak tetanic force was calculated as the difference between the maximum force during contraction and the force (or tension) measured 5 ms before the contraction. Resting tension was calculated as the difference in tension measured before a contraction during fatigue and before fatigue and was expressed as a percentage of the peak tetanic force measured in nonfatigued muscle.

**Action Potential Measurements**

Single action potentials and trains of action potentials were measured with conventional microelectrodes as described by Matar et al. (16). Briefly, action potentials were first measured while muscles were immersed in control solution, and muscles were then exposed for 30 min to 100 µM pinacidil before action potentials were measured. Microelectrodes (with tip potentials of <5 mV and tip resistance varying between 7 and 15 MΩ) and reference electrodes (tip resistance of 1 MΩ) were filled with 3 M KCl. Action potentials were elicited by passing small current between two fine platinum wires placed over the fibers on the muscle surface. Action potentials were recorded with a WPI electrometer (model M-707, Sarasota, FL) and digitized at a rate of 400 kHz for single action potentials and 33 kHz for action potential trains. Single action potentials were measured at a muscle length that gives maximum tetanic force (here referred to as unstretched muscle). Action potential trains were obtained with muscle fibers that were stretched to 1.5 times the optimal length for peak tetanic force to prevent movement artifacts. To avoid measurements from damaged fibers, trains of action potentials were accepted when the resting membrane potential before and the overshoot of the first action potential were within the range observed for single action potentials of unstretched muscles. For control muscle fibers, the ranges were between −67 and −94 mV for resting membrane potentials and between 15 and 42 mV for overshoots; these ranges being similar to those reported in previous studies involving mammalian muscle fibers (13, 33).

Resting potential was measured from the baseline of action potential. Overshoot was defined as the difference in potential between the peak and 0 mV. Half-repolarization time was measured as the time interval between the time of the overshoot and the time at which 50% of the repolarization had occurred. Action potential width was measured as the time interval at 50% of the amplitude. The first derivative (dV/dt) was obtained by calculating the slope over every 10 data points. Maximum rate of depolarization was measured.
as the peak of the first derivative during the depolarization phase.

**M Wave Measurements**

M waves were measured to determine the effects of pinacidil on action potentials during fatigue because 1) microelectrodes are easily broken or come out of muscle fibers when whole muscles are contracting, 2) any inhibition of muscle contraction by reducing Ca\(^{2+}\) release (e.g., with dantrolene) or by inhibiting actomyosin link formation (e.g., with butanedione monoxide) will affect ATP consumption and thus activation of K\(_{ATP}\) channels, and 3) most of the force recoveries occurred within 2–3 min (see Figs. 4 and 5), so action potentials measured after cessation of the stimulation (usually within 2–3 min) (16) do not really represent the fatigue condition. M wave measurements were carried out as described by Clausen and Overgaard (8) with separate nerve-muscle preparations for control and pinacidil-exposed muscles. Briefly, nerves were stimulated with suction electrodes while M waves were measured with two platinum wires. One wire (0.25 mm\(^2\)) was placed on the muscle surface, and the other wire was placed in the bath away from the muscle. M waves were recorded with a Grass P-15 AC amplifier and digitized at a rate of 33 kHz. M wave amplitude was calculated as the difference between the peak and baseline value and the area as the surface between the baseline and the upward peak.

**Statistical Analysis**

Split-plot ANOVA designs were used to determine significant differences for force, action potential, and M wave measurements because muscles were tested at all levels of time. ANOVA calculations were made with the general linear model (GLM) procedures of Statistical Analysis Software (SAS Institute). The least significant difference (LSD) was used to locate significant differences (26). Statistical differences were accepted at \( P < 0.05 \).

**RESULTS**

**Effects Of Pinacidil on Kinetics of Fatigue**

Mean peak tetanic forces of nonfatigued EDL muscle of wild-type and Kir6.2\(^{-/-}\) mouse were 32.3 ± 3.4 and 33.3 ± 3.2 N/cm\(^2\), respectively (no. of muscles, \( n = 5 \)). For soleus muscle, the values were 28.9 ± 1.9 and 24.4 ± 3.4 N/cm\(^2\), respectively (\( n = 5 \)). Pinacidil at 100 \( \mu \)M had no effect on tetanic force of nonfatigued muscles (data not shown). Thus there was no difference in peak tetanic force before fatigue between wild-type and Kir6.2\(^{-/-}\) muscles and between control and pinacidil-exposed muscles as previously reported (11, 16).

For EDL muscles, most of the decreases in peak tetanic force during fatigue occurred during the first 45 s (Fig. 1). For wild-type EDL muscles, the decrease in tetanic force during the first 30 s was significantly greater in the presence of 100 \( \mu \)M pinacidil (Fig. 1, A, B, and D). Pinacidil did not affect the final extent of fatigue, because peak tetanic forces of control and pinacidil-exposed muscles were no longer different after 45 s. The decreases in peak tetanic force of Kir6.2\(^{-/-}\) EDL were similar to those of wild-type EDL and were not affected by pinacidil (Fig. 1, C and E).

In wild-type soleus muscle, pinacidil also augmented the initial but not the final decrease in peak tetanic force (Fig. 2, A, B, and D). Furthermore, the pinacidil effect was the same whether soleus muscles were stimulated directly or via their motor nerve (Fig. 2D, inset). The pinacidil effect occurred later in soleus (30–75 s).

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**Fig. 1.** Pinacidil increases the rate of fatigue in wild-type but not in Kir6.2\(^{-/-}\) extensor digitorum longus (EDL) muscle. Tetanic contractions are shown from top to bottom after 0, 15, 30, 45, and 180 s of fatiguing stimulation (1 tetanic contraction every second) for control wild-type (A), 100 \( \mu \)M pinacidil-exposed wild type (B), and control Kir6.2\(^{-/-}\) (C) EDL. Horizontal bar, 50 ms; vertical bar, 20 N/cm\(^2\). Arrow in C indicates the increase in resting tension. Mean peak tetanic forces during fatigue are shown at every 15 s for wild-type (D) and Kir6.2\(^{-/-}\) (E) mice. Peak forces are expressed as % of the prefatigue force. Experimental temperature = 37°C. Control, ○, 100 \( \mu \)M pinacidil. Error bars represent SE of 5 muscles (not shown if smaller than symbols). *Mean peak tetanic force of pinacidil-exposed EDL muscle was significantly different from mean peak tetanic force of control EDL [ANOVA, least significant difference (LSD), \( P < 0.05 \)].
compared with EDL (15–30 s) muscles. The maximum difference in peak force between control and pinacidil-exposed muscles was twice as large in soleus (23% at 45 s) compared with EDL (11% at 30 s) muscles. The decreases in peak tetanic force of Kir6.2⁻/⁻ soleus during fatigue were not significantly different from those of wild-type soleus and were not affected by pinacidil (Figs. 2, C and E).

Resting tension increases when muscles fail to fully relax between contractions during fatigue. For wild-type EDL, the increase in resting tension was small and was never observed in the presence of pinacidil (Figs. 1, A and B, and 3A). Wild-type soleus (Figs. 2, A and B, and 3B) developed more resting tension than wild-type EDL. Again, no resting tension developed in wild-type soleus in the presence of pinacidil.

After fatigue, wild-type EDL muscles recovered 52% of their prefatigue peak tetanic force (Fig. 4, A and D),

Fig. 2. Pinacidil increases the rate of fatigue in wild-type but not Kir6.2⁻/⁻ soleus muscle. Tetanic contractions are shown (from top to bottom) after 0, 30, 60, 90, and 180 s of fatiguing stimulation for control wild-type (A), 100 μM pinacidil-exposed wild-type (B), and control Kir6.2⁻/⁻ (C) soleus. Horizontal bar, 50 ms; vertical bar, 10 N/cm². Arrow in A and C indicates the increase in resting tension. Mean peak tetanic forces during fatigue are shown for wild-type (D) and Kir6.2⁻/⁻ (E) soleus. Experimental temperature = 37°C. ○, Control; ○, 100 μM pinacidil; ○, ○, direct stimulation; ●, ●, nerve stimulation. Error bars represent SE of 5 muscles (not shown if smaller than symbol). *Mean peak tetanic force of pinacidil-exposed EDL muscle was significantly different from mean peak tetanic force of control EDL (ANOVA, LSD, P < 0.05).

Fig. 3. Pinacidil abolishes the development of resting tension during fatigue in wild-type EDL (A) and wild-type soleus (B) but not in Kir6.2⁻/⁻ EDL (C) and Kir6.2⁻/⁻ soleus (D) muscle. Mean increases in resting tension (relaxation tension) are expressed as % of the prefatigue peak tetanic force. Experimental temperature = 37°C. ○, Control; ○, 100 μM pinacidil. Error bars represent SE of 5 muscles (not shown if smaller than symbols). *Mean resting tension of pinacidil-exposed muscle was significantly different from mean resting tension of control muscle (ANOVA, LSD, P < 0.05). §Time at which the increase in mean resting tension became significantly different from the value at time 0 s (ANOVA, LSD, P < 0.05).
compared with 85% for pinacidil-exposed EDL (Fig. 4, B and D). For Kir6.2−/− EDL, force recovery was much less, reaching a maximum of 28% (Fig. 4, C and E), and was not affected by pinacidil. Wild-type soleus recovered 63% of its original force (Fig. 5, A and D). For these muscles, pinacidil increased the initial rate but not the final extent of force recovery (Fig. 5, B and D). Recovery of peak tetanic force in Kir6.2−/− soleus was similar to that in wild-type soleus and was not affected by pinacidil (Fig. 5E). Therefore, pinacidil increased the initial rate of fatigue, reduced resting tension during fatigue, and improved force recovery after fatigue in wild-type but not in Kir6.2−/− EDL and soleus muscles.

Effects of Pinacidil on Action Potentials

Single action potentials in nonfatigued muscle. Pinacidil at 100 μM had no effect on resting membrane potential and action potential half-repolarization time and width (Table 1), whereas it significantly reduced the overshoot and maximum rate of depolarization of...
wild-type muscles (Fig. 6). The pinacidil effects were reversible as the overshoot and maximum rate of depolarization returned back toward their original values on washout of pinacidil. Mean overshoots of Kir6.2−/− EDL and soleus and mean maximum rate of depolarization of Kir6.2−/− EDL were significantly lower than those of wild-type muscles. In Kir6.2−/− muscles, pinacidil had no effect on resting potential, half-repolarization time, width (Table 1), overshoot, or maximum rate of depolarization (Fig. 6).

Train of action potentials in nonfatigued muscles. Action potential trains were measured in muscles stretched to 1.5 times their optimal length to avoid movement artifacts. Mean resting membrane potentials of stretched EDL muscle fibers were ∼2–3 mV less (Table 2) than those from unstretched fibers (Table 1). For soleus muscles, the differences between stretched and unstretched fibers were slightly greater, being 5–6 mV. The membrane potential between action potentials constantly decreased during the 200-ms train (Fig. 7A). The mean depolarizations were 16 mV in wild-type EDL muscles (Fig. 7B) and 15 mV in Kir6.2−/− EDL muscles (Fig. 7C). The depolarizations were much less in soleus, being 9 and 6 mV for wild-type and Kir6.2−/− soleus, respectively. For all muscles, pinacidil did not significantly alter the depolarization during action potential trains.

Table 1. Pinacidil does not affect resting membrane potential and half-recovery time and width of single action potentials in unstretched muscle fibers

<table>
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<tr>
<th>Parameters</th>
<th>EDL</th>
<th>Wild type</th>
<th>Kir6.2−/−</th>
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<tr>
<td>Resting potential, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDL</td>
<td>C</td>
<td>−82.2 ± 2.1 (31/5)</td>
<td>−80.7 ± 1.7 (40/5)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>−79.2 ± 0.9 (36/5)</td>
<td>−79.5 ± 1.2 (37/5)</td>
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<tr>
<td></td>
<td>W</td>
<td>−79.9 ± 1.3 (42/5)</td>
<td>−77.0 ± 1.1 (43/5)</td>
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<tr>
<td>Soleus</td>
<td>C</td>
<td>−80.3 ± 1.1 (40/5)</td>
<td>−77.2 ± 1.2 (41/5)</td>
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<tr>
<td></td>
<td>P</td>
<td>−78.3 ± 1.6 (44/5)</td>
<td>−76.3 ± 2.6 (38/5)</td>
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<tr>
<td></td>
<td>W</td>
<td>−79.2 ± 1.0 (41/5)</td>
<td>−77.6 ± 1.5 (27/5)</td>
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<td>Half-recovery time, ms</td>
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<tr>
<td>EDL</td>
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<td></td>
<td>P</td>
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<tr>
<td></td>
<td>W</td>
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<td>0.270 ± 0.008</td>
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<tr>
<td></td>
<td>P</td>
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<td></td>
<td>W</td>
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<td>Width, ms</td>
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<tr>
<td>EDL</td>
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<td>0.370 ± 0.007</td>
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<td></td>
<td>P</td>
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<td></td>
<td>W</td>
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<tr>
<td>Soleus</td>
<td>C</td>
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<td>0.446 ± 0.005</td>
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<td></td>
<td>P</td>
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<tr>
<td></td>
<td>W</td>
<td>0.434 ± 0.006</td>
<td>0.429 ± 0.007</td>
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Data are means ± SE for nos. of fibers/muscles in parentheses. Experimental temperature was 33°C. EDL, extensor digitorum longus; C, control; W, 60 min of washout.

The first overshoots of action potential trains of wild-type stretched EDL and soleus muscle fibers (Fig. 8) were similar to those of single action potentials measured in unstretched fibers (Fig. 6). Mean action poten-

Table 2. Resting membrane potential of stretched muscle fibers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EDL</th>
<th>Wild type</th>
<th>Kir6.2−/−</th>
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<tr>
<td>Resting potential, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDL</td>
<td>C</td>
<td>−78.9 ± 1.1 (29/6)</td>
<td>−77.2 ± 0.7 (28/5)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>−76.0 ± 1.2 (28/6)</td>
<td>−77.2 ± 1.0 (26/5)</td>
</tr>
<tr>
<td>Soleus</td>
<td>C</td>
<td>−74.8 ± 1.2 (20/4)</td>
<td>−71.4 ± 1.1 (25/5)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>−71.5 ± 1.6 (17/4)</td>
<td>−71.4 ± 1.0 (20/5)</td>
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</table>

Data are means ± SE for nos. of fibers/muscles in parentheses. Experimental temperature was 33°C. C, control; P, 30 min at 100 µM pinacidil.
tial overshoots decreased during a train from 27 (first action potential) to 15 (last action potential; Fig. 8A) mV in wild-type EDL and from 29 to 16 mV in soleus (Fig. 8B). In the presence of pinacidil, the decreases were from 17 to 7 mV for EDL and from 16 to 7 mV for soleus. Thus for wild-type muscles overshoots were less in the presence of pinacidil throughout the train, but the decreases were slightly larger in control (12–13 mV) than in pinacidil-exposed (9–10 mV) muscles.

The overshoots of the first action potential in stretched Kir6.2−/− EDL fibers (Fig. 8C) were slightly less than those of unstretched fibers (Fig. 6), whereas there was no difference for Kir6.2−/− soleus fibers. Decreases in mean overshoots during the train were smaller in Kir6.2−/− EDL and soleus (6–9 mV) compared with those of wild-type muscles (12–13 mV), whereas pinacidil had no effect in Kir6.2−/− muscles.

Effects of pinacidil on M waves during fatigue. For these experiments, M waves were measured only in wild-type soleus because 1) the greatest effect of pinacidil on the rate of fatigue was in this muscle and 2) pinacidil had no effect in Kir6.2−/− muscles.

Fig. 7. Pinacidil does not affect membrane potential between action potentials during trains in nonfatigued muscle fibers. A: train of action potentials in wild-type EDL muscle. Horizontal bar, 40 ms; vertical bar, 40 mV. Changes in mean membrane potentials between action potentials in wild-type (B) and Kir6.2−/− (C) muscles are also shown. Stimulation frequencies: 200 (EDL) and 140 (soleus) Hz. O, control; △, EDL; ○, soleus; ○, control; ●, 100 μM pinacidil. Error bars represent SE (nos. of fibers/muscles are as indicated in Table 2). §Time at which the depolarization became significant during the train (ANOVA, LSD, P < 0.05).

Fig. 8. Pinacidil affects overshoot during action potential train in nonfatigued muscle fibers of wild-type but not Kir6.2−/− muscles. A: wild-type EDL (200 Hz). B: wild-type soleus (140 Hz). C: Kir6.2−/− EDL. D: Kir6.2−/− soleus. Experimental temperature = 33°C. Symbols: ○, Control; △, 100 μM pinacidil. Error bars represent SE (nos. of fibers/muscles are as indicated in Table 2). *Mean overshoot of pinacidil-exposed EDL muscle was significantly different from mean overshoot of control EDL (ANOVA, LSD, P < 0.05).
Train: The First 50 ms. M wave amplitudes during the first 50 ms of each train constantly decreased during fatigue. The decreases became significant within 15 s in the presence of pinacidil compared with 30 s in control soleus (Fig. 9B). The largest differences (19–20%) in M wave amplitudes between control and pinacidil-exposed soleus occurred between 30 and 45 s. Pinacidil did not affect the final extent of the decrease, because no significant difference was observed between control and pinacidil-exposed soleus after 120 s. Mean M wave areas of control soleus did not change significantly during the first 90 s of stimulation, whereas they constantly decreased in the presence of pinacidil, being 80 and 42% of the prefatigue values after 30 and 90 s, respectively (Fig. 9B). The differences between control and pinacidil-exposed soleus were much larger for the M wave areas than for amplitudes. For example, between 45 and 120 s the differences varied between 34 and 45%.

Train: The Last 150 ms. The decrease in mean M wave areas during the 200-ms train and the differences between control and pinacidil-exposed muscles were small in nonfatigued soleus (Fig. 10A). As fatigue developed, M wave areas decreased significantly during a train and the decreases were significantly greater in the presence of pinacidil within 45 s (Fig. 10B). After

Fig. 9. Pinacidil depresses M wave amplitude and area of wild-type soleus during fatigue. A: typical 200-ms train of M waves from nonfatigued soleus muscle. Amplitudes (B) and areas (C) during the first 50 s of the train (horizontal black bar in A) were averaged and are expressed as % of the average measured in nonfatigued muscle. Experimental temperature = 37°C. Control; ⊔, 100 μM pinacidil. Error bars represent SE of 5 muscles (not shown if smaller than symbols). *Mean amplitude or area of pinacidil-exposed soleus muscle was significantly different from mean amplitude or area of control soleus (ANOVA, LSD, P < 0.05). §Time at which mean amplitude or area became significantly different from the value at time 0 s (ANOVA, LSD, P < 0.05).

Fig. 10. Pinacidil affects train of M wave areas of wild-type soleus muscles during fatigue. Time during fatigue: 0 (A), 45 (B), 90 (C), and 180 (D) s. The 200-ms-long train was divided into four 50-ms periods. Areas of all M waves within each period were averaged and expressed as % of the averaged area during the first 50 ms. Experimental temperature = 37°C. Control; ⊔, 100 μM pinacidil. Error bars represent SE of 5 muscles. *Mean area of pinacidil-exposed soleus muscle was significantly different from mean amplitude of control soleus (ANOVA, LSD, P < 0.05). §Mean area became significantly <100% (ANOVA, LSD, P < 0.05).
90 s of stimulation, the mean M wave area of control soleus during the last 50 ms of the train (150–200 ms) was 32% less than those during the first 50 ms (0–50 ms), whereas for pinacidil-exposed muscles the decrease was 58% (150–200 ms; Fig. 10C). The differences in M wave areas between control and pinacidil-exposed muscles were still large after 180 s of stimulation (Fig. 10D). The changes in M wave amplitudes during the train and the differences between control and pinacidil-exposed muscles were similar to those for the areas (data not shown).

DISCUSSION

K\textsubscript{ATP} Channels: Effect on Force During Metabolic Stress

It has been hypothesized that K\textsubscript{ATP} channels are important in suppressing force during metabolic stress, such as fatigue, anoxia, or ischemia, to preserve ATP and prevent muscle function impairment. In regard to the effects of the channel on force, studies that used channel openers gave evidence for a suppression of force by K\textsubscript{ATP} channels during fatigue, anoxia, and metabolic inhibition, most studies failed to demonstrate under those conditions slower decreases in force on the addition of channel blockers (12, 14, 16, 28, 30). Even K\textsubscript{ATP} channel-deficient muscles from Kir6.2 \textsuperscript{−/−} mice have rates of fatigue that are similar to those of wild-type muscles (11). The only consistent effect of a lack of K\textsubscript{ATP} channel activity is greater resting tension (Fig. 3; Refs. 11, 12, 16). On the basis of these results, Gong et al. (11) suggested that one major effect of the K\textsubscript{ATP} channel is to reduce the development of resting tension.

A question was therefore raised in the introduction to this article as to whether channel openers reduce force via an activation of K\textsubscript{ATP} channels or via some nonspecific effects. This study gives strong evidence that the depression of action potential overshoot (Figs. 6 and 8), greater rate of fatigue (Figs. 1 and 2), abolition of resting tension during fatigue (Fig. 3), and improvement of force recovery after fatigue (Figs. 4 and 5) in wild-type mouse EDL and soleus muscles in the presence of pinacidil are all related to the activation of K\textsubscript{ATP} channels and not to some nonspecific effects. This conclusion is based on the fact that the pinacidil effects observed in wild-type muscles are not observed in Kir6.2 \textsuperscript{−/−} muscles that lack cell membrane K\textsubscript{ATP} channel activity. Thus K\textsubscript{ATP} channels depress force as originally hypothesized. We therefore propose that, once activated during a metabolic stress, including fatigue, K\textsubscript{ATP} channels reduce both force and resting tension.

The reasons why force appears unaffected when the activity of K\textsubscript{ATP} channel is abolished with channel blockers or by disrupting the Kir6.2 gene cannot be explained from the data of this study and are discussed in Role of K\textsubscript{ATP} Channels in Muscle. The following discussion focuses on the effects of K\textsubscript{ATP} channels on action potentials and their importance for the decrease in force.

Effect of K\textsubscript{ATP} Channels on Action Potentials

Nonfatigued muscle. Activating K\textsubscript{ATP} channels with pinacidil reduces the overshoot and maximum rate of depolarization of action potential (Fig. 6), which can be explained by an increase in K\textsuperscript{+} conductance allowing for a K\textsuperscript{+} efflux that counteracts the Na\textsuperscript{+} depolarization. However, contrary to the situation in cardiac muscle, an activation of K\textsubscript{ATP} channels has no effect on the half-repolarization time and duration (Table 1). Considering that pinacidil fails to increase K\textsuperscript{+} efflux in nonfatigued mouse EDL and soleus muscle (16), we suggest that the lower overshoot and slower depolarization when K\textsubscript{ATP} channels are activated results in smaller activation of the K\textsuperscript{+} delayed rectifiers, so that the total K\textsuperscript{+} conductance during the repolarization phase does not increase significantly. It is important to note, however, that contrary to the effect in nonfatigued muscles, during fatigue and metabolic inhibition K\textsubscript{ATP} channels significantly increase K\textsuperscript{+} efflux (7, 16) and shorten the duration of action potentials (12, 14).

The sarcolemma does not fully repolarize between action potentials during a train, resulting in a continuous depolarization (Fig. 7). The depolarization is most likely due to an accumulation of interstitial (and perhaps t tubular) K\textsuperscript{+} because the extent of sarcolemmal depolarization (Fig. 7) and K\textsuperscript{+} efflux (10, 20) during action potentials are less in soleus than in EDL muscle fibers. The overshoot also decreases during action potential trains (Fig. 8). One mechanism for the decrease in overshoot is an increase in the number of Na\textsuperscript{+} channels remaining inactivated as the sarcolemma repolarizes less and less between action potentials. Another mechanism is an accumulation of intracellular Na\textsuperscript{+}, which, by lowering the transmembrane Na\textsuperscript{+} concentration gradient, depresses the overshoot (6). However, the decrease in overshoot was not larger in EDL compared with soleus muscle fibers despite the fact that the sarcolemmal depolarization (Fig. 7) and Na\textsuperscript{+} influx (10, 20) are much greater in EDL muscle fibers. Perhaps the greater density of Na\textsuperscript{+} channels (19, 22) in EDL muscle fibers plays a significant role in preventing large decreases in overshoot in those muscle fibers.

Pinacidil did not affect the extent of depolarization during the action potential train, which is in agreement with the suggestion above that the total K\textsuperscript{+} conductance, and thus K\textsuperscript{+} efflux, during repolarization are not significantly increased in nonfatigued muscles. The only effect of activating K\textsubscript{ATP} channels with pinacidil is lower overshoots throughout the train compared with control conditions, whereas the extent of the overshoot decrease is unaffected.

Effects during fatigue. M waves were measured to determine the effects of K\textsubscript{ATP} channels on action potential trains during fatigue. The final extents of the decrease in M wave amplitude and area during fatigue were large but within the range of values reported in...
other studies for EDL, soleus, and diaphragm muscles (2, 21). In control wild-type soleus muscles, mean M wave amplitude decreases constantly during fatigue whereas the mean area does not change significantly for the first 90 s of fatiguing stimulation (Fig. 9). We therefore suggest that during that time action potential amplitude remains constant while the propagation along muscle fibers slows down to different extents in different fibers, resulting in M waves that are broader but of smaller amplitude. Decreases in action potential amplitude are expected to start after the first 90 s as both M wave amplitude and area decrease. In the presence of pinacidil, on the other hand, both M wave amplitude and area decrease constantly during fatigue, although the decrease is slightly less for the area. Furthermore, pinacidil accentuates the decrease in M wave area (Fig. 10) during a train as fatigue develops. We therefore suggest that during fatigue action potential amplitude decreases much sooner when K$_{ATP}$ channels are activated with pinacidil.

Effects of K$_{ATP}$ Channel on Force: Importance of Action Potentials

Nonfatigued muscle. Activation of K$_{ATP}$ channel with pinacidil fails to reduce peak force during twitches (30) and tetani (this study). Peak twitch force (and perhaps Ca$^{2+}$ release) of mouse EDL (32) and soleus (6) becomes depressed only when the overshoot decreases below 5 mV, a potential defined here as the critical overshoot. In the presence of pinacidil, the overshoot of single action potential remains well above 20 mV (Fig. 6). Similarly, in the presence of pinacidil, the overshoots at the end of 200-ms-long action potential trains is just above 5 mV (Fig. 8). Thus we conclude that for nonfatigued muscles the activation of K$_{ATP}$ channels with pinacidil does not suppress peak twitch and tetanic force because the effect on single action potentials and trains of action potentials are too small.

Effects during fatigue. In nonfatigued soleus muscle fibers, action potential overshoots decreases from 29 to 16 mV during a train. As discussed above, M wave measurements give no evidence for a decrease in action potential amplitude in control soleus during the first 90 s of fatiguing stimulation. Thus action potential overshoots are expected to decrease below the critical overshoot some time after 90 s of stimulation. On the other hand, action potential overshoots of nonfatigued pinacidil-exposed soleus fibers are much closer to the critical overshoot as they decrease from 16 to 7 mV. M wave measurements suggest that action potential amplitude decreases significantly within 30 s of stimulation. It is then more than likely that action potential overshoots of pinacidil-exposed fibers fall below the critical overshoot early during fatigue. Furthermore, the time at which the pinacidil effects on M waves become significant during fatigue corresponds to the time when the effect on peak tetanic force also becomes significantly greater than those in control soleus (Figs. 2D, 9, 10). It is thus suggested that in the presence of pinacidil action potential overshoot rapidly falls below the critical overshoot during fatigue and that this effect is responsible for the faster rate of fatigue compared with control muscles.

Role of K$_{ATP}$ Channels in Muscle

If K$_{ATP}$ channels contribute to the decrease in force during fatigue, in the absence of channel activity the decrease in force should be slower, an effect that most studies have so far failed to demonstrate. A lack of K$_{ATP}$ channel activity results in severe membrane depolarization of skeletal muscle fibers during metabolic inhibition (12) and in patients suffering of hypokalemic periodic paralysis (15, 23, 27). More importantly, these depolarizations are associated with large decreases in force. Perhaps the depolarizations inactivate Na$^+$ channels, resulting in smaller action potentials, Ca$^{2+}$ release, and thus force. It is therefore suggested that slower decreases in force are not observed during fatigue, anoxia, and metabolic inhibition when the activity of K$_{ATP}$ channels is abolished with channel blockers or by disrupting the Kir6.2 gene because the capacity to generate action potentials and thus force is impaired by large membrane depolarization. In cardiac muscles large membrane depolarization affects the activity of L-type Ca$^{2+}$ channels (34) and Na$^+$/Ca$^{2+}$ exchangers (1), causing increases in intracellular Ca$^{2+}$ concentration and diastolic pressure. It remains to be determined whether depolarization of the cell or t-tubular membrane in the absence of K$_{ATP}$ channel activity has similar effects in skeletal muscle.

In summary, this study showed that pinacidil does not affect the action potentials and fatigue kinetics in muscles of the Kir6.2$^{-/-}$ mouse, suggesting that all the effects of pinacidil observed in wild-type muscles during fatigue are due to an activation of K$_{ATP}$ channels. We thus suggested that one major role of the K$_{ATP}$ channel is to depress action potentials, which eventually reduces force development. The K$_{ATP}$ channel is also important in preventing the development of resting tension during fatigue and in improving force recovery after fatigue, especially in fast-twitch muscles like mouse EDL.

DISCLOSURES

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