An abnormal ratio between Na⁺ and K⁺ conductances seems to be the cause for the depolarization and paralysis of skeletal muscle in primary hypokalemic periodic paralysis. Recently we have shown that the "K⁺ channel opener" cromakalim hyperpolarizes mammalian skeletal muscle fibers. Now we have studied the effects of this drug on the twitch force of muscle biopsies from normal and diseased human skeletal muscle. Cromakalim had little effect on the twitch force of normal muscle whereas it strongly improved the contraction force of fibers from patients suffering from hypokalemic periodic paralysis. Recordings of intracellular K⁺ and Cl⁻ activities in human muscle and isolated rat soleus muscle support the view that cromakalim enhances the membrane K⁺ conductance (gK⁺). These data indicate that "K⁺ channel openers" may have a beneficial effect in primary hypokalemic periodic paralysis.

Key words: cromakalim • inotropic effect • intracellular ion activities • skeletal muscle, human • periodic paralysis


ENHANCEMENT OF K⁺ CONDUCTANCE IMPROVES IN VITRO THE CONTRACTION FORCE OF SKELETAL MUSCLE IN HYPOKALEMIC PERIODIC PARALYSIS

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There is good evidence that a depolarization of the muscle fiber membrane causes the loss of contractility in primary hypokalemic periodic paralysis (for review see Refs. 5 and 15). The pathophysiological basis for this disease seems to be an abnormal ratio in the relative conductances of Na⁺ and K⁺.₇,₁₃,₁₅ Investigations on excised external intercostal muscle fibers revealed an unusual high Na⁺ conductance and fiber depolarization in primary hypokalemic periodic paralysis.₁₄ Based on these findings one would expect that an increase in membrane K⁺ conductance (gK⁺) should induce a membrane repolarization and an increase in muscle force in the periodic paralysis.

Recently, we have found that drugs known to enhance gK⁺ in smooth and cardiac muscle (for review see Refs. 4 and 8) exert a similar action also on human skeletal muscle.₁₂,₁₈ Here we report about the effects of the "K⁺ channel opener" cromakalim on the twitch force of normal and diseased isolated human skeletal muscle. The data show that cromakalim strongly improves the muscle strength of fibers from patients with primary hypokalemic periodic paralysis, whereas no effect on twitch force was found in control muscle. The experiments were supplemented with measurements of intracellular K⁺ and Cl⁻ activities in human and rat muscle fibers in order to gain more insight into the pathophysiology of the disease and the mechanisms underlying the effects of cromakalim on skeletal muscle.

MATERIALS AND METHODS

The experiments were performed on biopsies of human skeletal muscle.₁₀,₁₁ Specimens of fiber
segments (4–6 cm long) were dissected from bi-
iceps brachii, deltoid, vastus medialis or vastus lat-
eralis muscles under local anesthesia. Such fiber
segments were obtained from (1) patients who
consented that a muscle biopsy be carried out for
the in vitro diagnosis of susceptibility to malignant
hyperthermia; (2) patients who required a muscle
biopsy for clinical diagnosis; and (3) patients with
a known neuromuscular disease who consented to
have a muscle biopsy for experimental reasons
only. All procedures were in accordance with the
Helsinki convention and the Ethics Committee of
the Technical University of Munich. Intracellular
ion activities were also measured in fibers from
isolated rat soleus muscle.

The preparations were transferred to a per-
spex chamber (volume 2 mL, flow rate 6.5 mL/
min) and superfused at 36°C with a solution of the
following composition

\[
\text{NaCl } 107.7; \quad \text{CaCl}_2 \ 1.53; \quad \text{KCl} \ 3.48; \quad \text{MgSO}_4 \ 0.69; \quad \text{NaHCO}_3 \ 26.2; \quad \text{NaH}_2\text{PO}_4 \ 1.67; \quad \text{Na-glucuronate} \ 9.64; \quad \text{glu}-
cose \ 5.5; \quad \text{succrose} \ 7.6 \quad \text{(bubbled with 95\% O}_2/5\% \ \text{CO}_2; \ \text{pH} \ 7.4). \quad \text{Micro-electrodes were drawn on a}
\]

Brown Flaming micropipette puller (Sutter Instru-
tments, San Rafael, CA, USA). Single-barreled
electrodes had tip diameters below 0.2 μm (filling
solution 3 mol/L KCl, resistance 40–60 MOhm).
The methods for the construction and calibration
of the double-barreled ion-sensitive micro-elec-
trodes (tip size 1.0–1.5 μm) are described in detail
elsewhere.

The following combinations of ion-
exchanger, backfilling solution, and reference
solution were used: K+-sensitive micro-electrodes
(Corning 477317, 200 mmol/L KCl, 1 mol/L
\text{Mg}^{2+} \ \text{acetate); Cl}\text{-sensitive micro-electrodes (WP}
\text{Instruments IE-170, 200 mmol/L KCl, 0.5 mol/L}
K\text{+ sulfate). For the measurement of contrac-
tion force, a muscle fiber bundle was suspended
in the perspex chamber with one end fixed and
the other end fastened to a strain gauge (Hot-
tinger Baldwin, Darmstadt, FRG). Silver plates
on both sides of the preparation were used
for direct (“field”) stimulation. Supramaximal sin-
gle square voltage pulses (2 msec duration,
100 V stimulus strength) were applied every 10
sec.

Drugs were applied via the bathing solution. Glibenclamide was obtained from Hoechst (Frank-
furt, FRG); cromakalim (BRL 34915) was a gift
from Beecham Pharmaceuticals (Gronau, FRG).
Stock solutions (100 mmol/L cromakalim, 10
mmol/L glibenclamide) were prepared in dimethyl
sulfoxide (DMSO). DMSO did not mimic the ef-
effects of cromakalim or glibenclamide.

RESULTS

Effects of Cromakalim on Intracellular K\textsuperscript+ Activity.
The hyperpolarizing effect of cromakalim on hu-
man and also rat skeletal muscle was accompanied
by a small decrease in the intracellular K\textsuperscript+ activity
(a\textsubscript{K\textsuperscript{+}}). Fig. 1 gives typical examples of the measure-
ments of a\textsubscript{K\textsuperscript{+}} in human and rat skeletal muscle
cells. The baseline level of a\textsubscript{K\textsuperscript{+}} of this control hu-
man muscle fiber, which had a resting membrane
potential (E\textsubscript{m}) of −70 mV, was 95 mmol/L (Fig.
1A). Addition of cromakalim to the bathing solu-
tion resulted in a membrane hyperpolarization of
15 mV and a decrease in a\textsubscript{K\textsuperscript{+}} from 95 to about 93
mmol/L. The voltage difference between the K\textsuperscript{+}-
sensitive and the reference electrode (E\textsubscript{Diff}) was
0.5 mV. This amplitude is only a little higher than
the noise level of our K\textsuperscript{+}-sensitive micro-elec-
trodes. Therefore, cromakalim-induced changes
in a\textsubscript{K\textsuperscript{+}} were close to the detection limit of the
method. Fig. 1B shows a similar experiment in a

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Effects of cromakalim on intracellular K\textsuperscript{+} activity (a\textsubscript{K\textsuperscript{+}}) and membrane potential (E\textsubscript{m}) of a fiber in a normal human skeletal muscle segment (A) and in an isolated rat soleus muscle (B). E\textsubscript{Diff} is the difference between the voltage reading of the K\textsuperscript{+}-sensitive and the reference barrel of the double-barreled ion-sensitive micro-electrode. Cromakalim was applied via the bathing solution during the times indicated by the bars.}
\end{figure}
muscle fiber from an isolated rat soleus muscle. In this case, cromakalim induced a membrane hyperpolarization of 10 mV; aK decreased from 93 to about 91 mmol/L. In summary, application of cromakalim resulted in a small decrease in aK of human and rat skeletal muscle fibers. A quantitative analysis of this effect is summarized in Table 1.

**Effects of Cromakalim on Intracellular Cl⁻ Activity.** Cromakalim induced a decrease in intracellular Cl⁻ activity (aCl) of human and rat skeletal muscle. Figure 2 illustrates an example of this effect. A relatively blunt double-barrelled Cl⁻-sensitive micro-electrode (tip diameter 1.5 μm) was used in this experiment (rat soleus muscle). Fiber impalements with such electrodes resulted in recordings of low membrane potentials and relatively high aCl. The effect of cromakalim on these fibers was a strong hyperpolarization (~20 mV or more) accompanied by a decrease in aCl. Table 1 summarizes the recordings of aCl in rat and human fibers before and during the application of cromakalim.

**Hypokalemic Periodic Paralysis.** Muscle specimens were obtained from 4 patients with hypokalemic periodic paralysis. Fiber segments of these muscles had membrane potentials less negative than control values in solutions with normal extracellular K⁺ concentration (3.5 mmol/L). The average membrane potential obtained with single-barrelled electrodes in 16 fibers of 4 control muscles was −80.7 ± 5.3 mV (mean ± SD); 17 fibers from 4 patients with hypokalemic periodic paralysis had an average Em of −71 ± 6.2 mV. The lower membrane potentials of the affected fibers are, however, not due to a decreased K⁺ equilibrium potential (EK) since an aK of 93.1 ± 1.6 mmol/L (mean ± SD, n = 5 fibers in 2 muscles) was found.

Cromakalim induced a strong hyperpolarization in muscle fibers from patients with hypokalemic periodic paralysis. This is illustrated in Fig. 3. The fiber was impaled with a double-barrelled K⁺-sensitive micro-electrode. At the beginning of the recording (solution with 3.5 mmol/L K⁺), a resting potential of −62 mV and an E₉ of −95 mV was found. The membrane depolarized further and the difference between Em and E₉ increased when [K⁺], in the bathing solution was reduced to 1 mmol/L. (A membrane depolarization upon lowering of [K⁺], is a characteristic observation in isolated skeletal muscle fibers from patients with hypokalemic periodic paralysis.) In this situation, the application of cromakalim resulted in a reversible membrane hyperpolarization of 35 mV. Later on, [K⁺], was raised to 3.5 mmol/L. This produced only a slight increase in Em, whereas aK returned to its baseline level. Similar observations were made on another 4 fibers from 2 patients.

<table>
<thead>
<tr>
<th>Type of muscle</th>
<th>E₉ (mV)</th>
<th>ΔEm (mV)</th>
<th>aK (mmol/L)</th>
<th>ΔaK (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, soleus (8;2)</td>
<td>−72.2 ± 5.7</td>
<td>−6.8 ± 1.7</td>
<td>88.9 ± 4.6</td>
<td>−2.7 ± 0.6</td>
</tr>
<tr>
<td>Human, control (12;2)</td>
<td>−73.3 ± 4.8</td>
<td>−10.7 ± 5.3</td>
<td>96.1 ± 6.7</td>
<td>−2.8 ± 1.1</td>
</tr>
<tr>
<td>Rat, soleus (11;3)</td>
<td>−71.0 ± 6.2</td>
<td>−12.2 ± 3.5</td>
<td>9.2 ± 2.1</td>
<td>−1.3 ± 0.8</td>
</tr>
<tr>
<td>Human, control (6;2)</td>
<td>−72.5 ± 7.3</td>
<td>−15.3 ± 10.4</td>
<td>8.0 ± 0.6</td>
<td>−2.0 ± 1.4</td>
</tr>
</tbody>
</table>

*Mean values ± SD; the number of fibers and muscle preparations are given in parentheses.
Twitch Force. The twitch force of segments of human skeletal muscle was tested by direct stimulation of the muscle bundles (stimulus strength 100 V, duration 2 msec). These experiments revealed a differential effect of cromakalim. The drug preferentially increased the twitch force of fiber bundles from patients with hypokalemic periodic paralysis but not from controls. An example of such recordings is illustrated in Fig. 4. The registration in part A shows the effect of cromakalim on the twitch force of a control muscle. There was a relatively high contraction force in the standard solution. Application of the drug into the bathing solution did not increase the twitch force. In contrast, fiber bundles from patients with hypokalemic periodic paralysis exerted little (sometimes no) twitch force in the normal bathing solution. However, cromakalim increased the contraction force of these muscles several times (Fig. 4B). This effect was only slowly reversible. After a 10-minute application of cromakalim, it took 30 minutes or more before the contraction force returned to the original value. All the measurements of twitch force before and during the application of cromakalim are summarized in Fig. 5. The preferential effect of cromakalim on the diseased muscle is clearly visible (note the logarithmic scale). Cromakalim had this effect in a bathing solution containing 3.5 mmol/L [K+]e as well as 1 mmol/L [K+]e.

**Effects of Glibenclamide.** We have shown previously that tolbutamide antagonizes the effect of cromakalim on human skeletal muscle. Now we have found that glibenclamide, another sulfonyleurea compound, also blocks the effects of cromakalim. Fig. 6 illustrates this antagonism in a fiber from a patient suffering from hypokalemic periodic paralysis. The resting potential of this fiber in a bathing solution of 1 mmol/L [K+]e was -50 mV. Cromakalim induced a membrane hyperpolarization of more than 40 mV. However, this effect was completely suppressed after 1 μmol/L glibenclamide was added to the cromakalim-containing solution. A similar observation was made on another 6 fibers, on which the effects of glibenclamide were explored. In four other experiments the effects of glibenclamide on the contraction force of normal and diseased human skeletal muscle biopsies were tested. We observed that glibenclamide itself had no effect on the twitch force. However, it completely suppressed or prevented the contraction force–facilitating effect of cromakalim on fiber bundles from patients with hypokalemic periodic paralysis (not illustrated).
DISCUSSION

The present study shows that cromakalim (a) hyperpolarizes normal and diseased human skeletal muscle fibers and (b) improves contraction force of fiber bundles from patients with primary hypokalemic periodic paralysis. Cromakalim belongs to a new class of drugs, called “K+ channel openers”, which activate K+ channels in a variety of smooth muscle cells (for review see Refs. 4 and 5), in cardiac muscle cells6,17 and in human skeletal muscle cells6,17. One of the consequences of such an effect should be an efflux of intracellular K+ ions and a decrease in the intracellular K+ activity.

In fact, the measurements with K+-sensitive micro-electrodes in the present study revealed a diminution of aK+. This finding, therefore, supports the idea that cromakalim enhances gK+ also in skeletal muscle. Previously, this has been demonstrated by means of current-voltage relationships.18 The change in aCl- seen during the application of cromakalim can be explained by the high Cl- conductance of the muscle fiber membrane. This conductance leads to a passive Cl- efflux when the membrane hyperpolarizes. Effects of cromakalim on fluxes of sodium and calcium ions have not been investigated in the present study. However, it is unlikely that a decrease in the Na+ conductance underlies the membrane hyperpolarization seen in the presence of cromakalim. Such an effect should result in an increase rather than a decrease of aK+. Effects of cromakalim on the membrane Ca2+ conductance or Ca2+ transport mechanisms are possible and should be investigated in future experiments.

The experiments in which the effects of glibenclamide were explored give an indication regarding the type of K+ channel activated by cromakalim. Glibenclamide seems to be a specific antagonist of ATP-dependent K+ channels7,19 and it antagonizes the effect of cromakalim in cardiac muscle6,17 and also in human skeletal muscle (present study). It seems very likely, therefore, that cromakalim hyperpolarizes human skeletal muscle by the opening of otherwise “silent”, ATP-
dependent K+ channels. Other K+ channels in skeletal muscle, like the α-channel, delayed rectifier, and calcium-activated potassium channels are not antagonized by glibenclamide. Therefore, we find it unlikely that the conductance of these channels is modulated by cromakalim although no direct experimental evidence exists.

Cromakalim improved the contraction force of muscle fibers from patients with primary hypokalemic periodic paralysis. Two mechanisms are probably involved in this effect. First, slow inactivation of Na+ channels is a possible mechanism for reduced excitability in muscle fibers with low membrane potentials, e.g. in primary hypokalemic periodic paralysis. This process is especially important because it operates around the resting membrane potential. Consequently, the observed repolarization could restore simultaneously normal K+ channels and of the transduction protein does not operate in the voltage range of the normal K+ equilibrium potential. It is understandable, therefore, that cromakalim does not improve the contraction force of normal muscle fibers.

Two main conclusions can be drawn from the present study. First, the data support the view that a low membrane potential is the cause for paralysis in primary hypokalemic periodic paralysis. Secondly, drugs from the class of "K+ channel openers" may have clinical relevance in the treatment of primary hypokalemic periodic paralysis. Since these drugs activate K+ channels in a variety of tissues (e.g., vascular smooth muscles) the therapeutic use may be limited by their side effects. Nevertheless, the concentrations used in our in vitro study are very likely above the concentrations necessary to exert an effect in a well perfused muscle. There is no ideal diffusion in muscle biopsies and the drug concentration in the center of the muscle tissue is below the concentration in the bathing solution.

The next question concerns the different effect of cromakalim on the contraction force of normal and diseased muscle. The most likely explanation for this finding is the difference in membrane potential. Diseased fibers are depolarized in spite of a normal K+ equilibrium potential. In such a situation, "K+ channel openers" exert a strong hyperpolarizing effect (and an increase in contraction force, see above). The resting potentials of normal fibers, on the other hand, are close to the K+ equilibrium potential and cromakalim has little effect. Furthermore, inactivation of Na+ channels and of the transduction protein does not operate in the voltage range of the normal K+ equilibrium potential. It is understandable, therefore, that cromakalim does not improve the contraction force of normal muscle fibers.

REFERENCES


