

EJP 20690

Short communication

K⁺ channel openers suppress myotonic activity of human skeletal muscle in vitro

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Received 29 June 1990, accepted 10 July 1990

Isolated fibre bundles from myotonic human skeletal muscle showed after-contractions and spontaneous mechanical activity. The K⁺ channel openers cromakalim (10–100 μmol/l) and EMD 52962 (1–10 μmol/l) completely suppressed these abnormalities in mechanical activity. Voltage-clamp experiments revealed that cromakalim (100 μmol/l) increased the membrane K⁺ conductance of isolated, non-myotonic human skeletal muscle fibres 4-fold; Cl⁻ conductance was not altered. The data show that myotonia is suppressed by an increase in membrane K⁺ conductance.

Myotonia; Skeletal muscle (human); K⁺ channel openers; Voltage clamp; Membrane conductance

1. Introduction

Potassium channel openers enhance the membrane conductance of human skeletal muscle (Spuler et al., 1989). The resulting membrane hyperpolarization may be of therapeutic benefit in muscle diseases in which membrane depolarization causes inexcitability (e.g. hypokalaemic periodic paralysis, see Grafe et al., 1990). Another important symptom of diseases skeletal muscle is myotonia. This phenomenon is characterized by delayed skeletal muscle relaxation and spontaneous muscle contractions. It seems possible that an increase in K⁺ conductance may stabilize the membrane of hyperexcitable fibres. We therefore analysed the effects of cromakalim and EMD 52962, two potent K⁺ channel openers (De Peyer

et al., 1989; Quast and Cook, 1989), on the mechanical activity of fibre bundles from myotonic human skeletal muscle. These experiments were supplemented by a voltage-clamp analysis of the effects of cromakalim on membrane conductance.

2. Materials and methods

Experiments were performed on biopsyspecimens of human skeletal muscle (see Lehmann-Horn and Iaizzo, 1990; Spuler et al., 1989). In the present study, fibre segments were obtained from (a) four patients with myotonia congenita, (b) four patients suffering from myotonic dystrophy and (c) one patient with hyperkalaemic periodic paralysis. One end of a fibre bundle was fixed in a perspex chamber and the other end fastened to a strain gauge (Hottinger Baldwin, Darmstadt, F.R.G.). The preparation was placed between two silver plates which were used for direct ('field') stimulation. Supramaximal single square voltage

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pulses (2 ms duration, 100 V stimulus strength) were applied every 10 s. The standard bathing solution (Bretag, 1969) contained (in mmol/l): NaCl 107.7; KCl 3.48; CaCl₂ 1.53; MgSO₄ 0.69; NaHCO₃ 26.2; NaH₂PO₄ 1.67; Na-gluconate 9.64; glucose 5.5; sucrose 7.6 (gassed with 95% O₂, 5% CO₂, pH 7.4). The Cl⁻-free solution was made by replacing NaCl and KCl with the respective methane sulfonate salts, and CaCl₂ with Ca-gluconate. Drugs were added to the bathing solution. Stock solutions of cromakalim (Beecham Pharmaceuticals) and EMD 52962 (E. Merck Pharmaceuticals) were prepared in dimethylsulfoxide (DMSO). DMSO did not mimic the effects of either cromakalim or EMD 52962.

Voltage-clamp experiments were performed with three microelectrodes (Lehmann-Horn et al., 1981). Voltage-clamp step cycles were used, starting from a holding potential of -80 mV and covering a voltage range from -120 to -56 mV. Membrane potential, potential difference and clamp current were pulse-code-modulated and stored on a magnetic tape (Johne and Reilhofer, München, F.R.G.). With a PDP 11/23 computer (Digital Equipment Corporation, Maynard, MA, U.S.A.) the current density-membrane potential relationships were calculated using an algorithm of Adrian and Marshall (1971). An AT-personal computer was used to reduce and average the data further.

3. Results

In a first series of experiments, the effects of the K⁺ channel openers were tested on (a) stimulus-induced single muscle twitches and (b) spontaneous mechanical activity of unstimulated preparations. Figure 1 illustrates data obtained with a fibre bundle from a patient with myotonia congenita. Similar experiments were done with biopsies from patients with myotonic dystrophy. In myotonic preparations, an initial muscle twitch of approximately normal force and duration was followed by after-contractions lasting for several seconds (fig. 1A). The twitch force of these after-contractions was variable, and their magnitudes were only about 1-10% of that of the initial twitch.

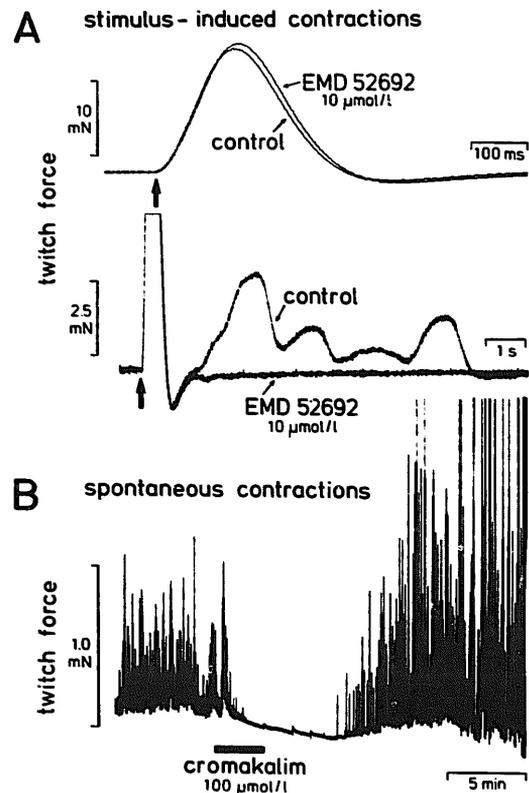


Fig. 1. Effects of K⁺ channel openers on myotonic activity of a skeletal muscle fibre bundle from a patient with myotonia congenita. Cromakalim and EMD 52962 were applied via the bathing solution. (A) Examples of stimulus-induced muscle contractions (field stimulation with 2-ms duration and 100-V stimulus strength, indicated by arrows) obtained in normal bathing solution (control) and in the presence of EMD 52962 are superimposed. The upper panel shows the initial twitch, the lower one the after-contractions (note increase in twitch force amplification and slower time base). (B) Continuous recording of spontaneous mechanical activity.

EMD 52962 at concentrations of 1-10 µmol/l completely suppressed the after-contractions. The force of the initial twitch, however, was only slightly increased. Similar observations were made with cromakalim (10-100 µmol/l).

In another series of experiments, the effects of EMD 52962 and cromakalim on spontaneous mechanical activity of unstimulated preparations were investigated (fig. 1B). In normal bathing solution, spontaneous, irregular muscle contractions of variable twitch force were observed. This myotonic activity was completely suppressed soon after the addition of cromakalim (10-100 µmol/l)

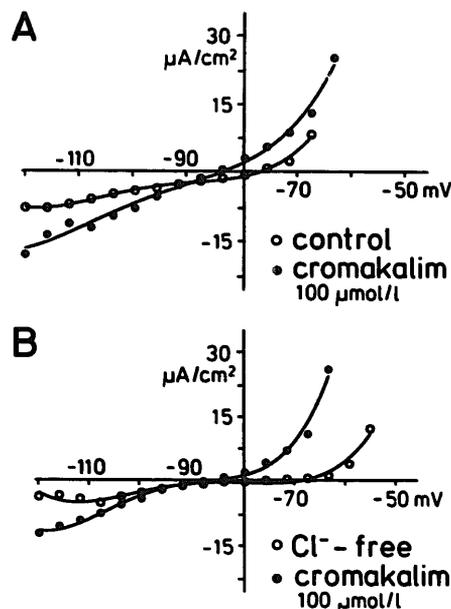


Fig. 2. Current density-voltage relationships in control (Bretag's; A) and Cl^- -free solutions (B) with and without cromakalim ($100 \mu\text{mol/l}$). The muscle fibre bundle was from a patient with hyperkalaemic periodic paralysis. Averaged data from measurements in 29 fibres are shown. One current-voltage relationship was determined in each fibre. All solutions contained tetrodotoxin (TTX, $1 \mu\text{mol/l}$). For further information see text.

to the bathing solution. Similar effects were also observed with EMD 52962 ($1\text{-}10 \mu\text{mol/l}$). Upon withdrawal of the K^+ channel openers, a prompt recovery of myotonic activity was seen. In the post-drug period, the frequency and twitch force of spontaneous contractions transiently increased as compared to control. This effect is probably related to the hyperpolarizing effect of K^+ channel openers (Spuler et al., 1989), which may remove the slow Na^+ inactivation (Ruff et al., 1988). Therefore, the subsequent depolarization in the recovery period leads to more myotonic activity.

The effects of cromakalim ($100 \mu\text{mol/l}$) on the current density-voltage relationship was determined in 29 fibres of a fibre bundle taken at biopsy from a patient with hyperkalaemic periodic paralysis. The data are illustrated in fig. 2. The specific membrane conductance was determined by calculating the slope of the curves at the resting potential. In the control solution (Bretag's + TTX, $1 \mu\text{mol/l}$), we calculated a total membrane con-

ductance (g_m) of $265 \mu\text{S/cm}^2$ (mean of 10 observations), a potassium conductance (g_K , measured in Cl^- -free solution; $n = 6$) of $84 \mu\text{S/cm}^2$ and a chloride conductance ($g_{\text{Cl}} = g_m - g_K$) of $181 \mu\text{S/cm}^2$. In the solution containing $100 \mu\text{mol/l}$ cromakalim, g_m increased 2-fold to $531 \mu\text{S/cm}^2$ ($n = 6$; fig. 2A) and g_K increased 4-fold to $339 \mu\text{S/cm}^2$ ($n = 7$; fig. 2B). The absolute value of g_{Cl} did not change ($192 \mu\text{S/cm}^2$). These results confirm the suggestion that cromakalim increases g_K without changing g_{Cl} .

4. Discussion

Our data show that the K^+ channel openers cromakalim and EMD 52962 suppress myotonic activity in fibre bundles from diseased human skeletal muscle. Myotonic activity was never observed in biopsies of skeletal muscle from control persons. The anti-myotonic effect of cromakalim and EMD 52962 is therefore specific for diseased fibres and is not simply due to a restoration of biopsy damage. An increase in membrane K^+ conductance is probably the mechanism underlying the suppression of stimulus-induced electrical after-discharges and of spontaneous action potentials. However, direct intracellular electrophysiological recordings from myotonic fibres were not possible because of spontaneous muscle contractions. However, previous studies with normal and diseased human skeletal muscle (Spuler et al., 1989; Grafe et al., 1990) and the present experiments (see fig. 2) clearly showed an enhancement of membrane K^+ conductance by cromakalim. A decrease in Na^+ conductance, as an alternative mechanism for an anti-myotonic effect, seems unlikely since only the after-contractions following electrical stimulation and not the initial muscle twitch were suppressed by cromakalim and EMD 52962 (see fig. 1A).

Acknowledgements

This work was supported by the Wilhelm Sander Stiftung. The authors would like to thank Drs. Ch. Franke and W. Klein (Department of Neurology, Technical University of Munich)

for the preparation of the muscle biopsies, Ms. C. Müller for technical and secretarial assistance, and Dr. K. Feasey and Dr. A.A. Parsons for reading the manuscript. We are grateful to Beecham Pharmaceuticals (Gronau, F.R.G.) for a gift of cromakalim (BRL 34915) and to Dr. Wurziger (Merck, Darmstadt, F.R.G.) for kindly supplying us with EMD 52962.

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