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

MUSCLE & NERVE 13:451–457 1990

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^+.[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.

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Acknowledgments: We thank Ms. C. Müller, Ms. G. Meier and Ms. E. Höhne for technical and secretarial assistance. This work was supported by the Wilhelm Sander Stiftung (PG).

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Accepted for publication July 11, 1989.

CCC 0148-639X/90/050451-07 $04.00

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 biceps brachii, deltoid, vastus medialis or vastus lateralis 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 perspex chamber (volume 2 mL, flow rate 6.5 mL/min) and superfused at 36°C with a solution of the following composition[3] (in mmol/L): NaCl 107.7; $CaCL_2$ 1.53; KCl 3.48; $MgSO_4$ 0.69; $NaHCO_3$ 26.2; NaH_2PO_4 1.67; Na-glucuronate 9.64; glucose 5.5; sucrose 7.6 (bubbled with 95% O_2/5% CO_2; pH 7.4). Micro-electrodes were drawn on a Brown Flaming micropipette puller (Sutter Instruments, San Rafael, CA, USA). Single-barrelled 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-barrelled ionsensitive micro-electrodes (tip size 1.0–1.5 μm) are described in detail elsewhere.[1] 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 Mg^{2+} acetate); Cl^--sensitive micro-electrodes (WP Instruments IE-170, 200 mmol/L KCl, 0.5 mol/L K^+ sulfate). For the measurement of contraction force, a muscle fiber bundle was suspended in the perspex chamber with one end fixed and the other end fastened to a strain gauge (Hottinger Baldwin, Darmstadt, FRG). Silver plates on both sides of the preparation were used for direct ("field") stimulation. Supramaximal single 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 (Frankfurt, 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 effects of cromakalim or glibenclamide.

RESULTS

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

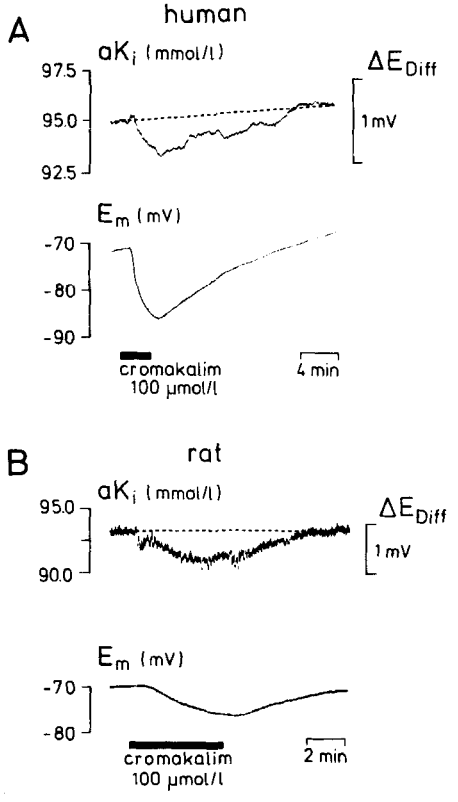

FIGURE 1. Effects of cromakalim on intracellular K^+ activity (aK_i) and membrane potential (E_m) of a fiber in a normal human skeletal muscle segment **(A)** and in an isolated rat soleus muscle **(B).** E_{Diff} is the difference between the voltage reading of the K^+-sensitive and the reference barrel of the double-barrelled ion-sensitive micro-electrode. Cromakalim was applied via the bathing solution during the times indicated by the bars.

muscle fiber from an isolated rat soleus muscle. In this case, cromakalim induced a membrane hyperpolarization of 10 mV; aK_i decreased from 93 to about 91 mmol/L. In summary, application of cromakalim resulted in a small decrease in aK_i 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_i) 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_i. The effect of cromakalim on these fibers was a strong hyperpolarization (−20 mV or more) accompanied by a decrease in aCl_i. Table 1 summarizes the recordings of aCl_i 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 E_m of −71 ± 6.2 mV. The lower membrane potentials of the affected fibers are, however, not due to a decreased K^+ equilibrium potential (E_K) since an aK_i of 93.1 ± 1.6 mmol/L (mean ± SD, n = 5 fibers in 2 muscles) was found.

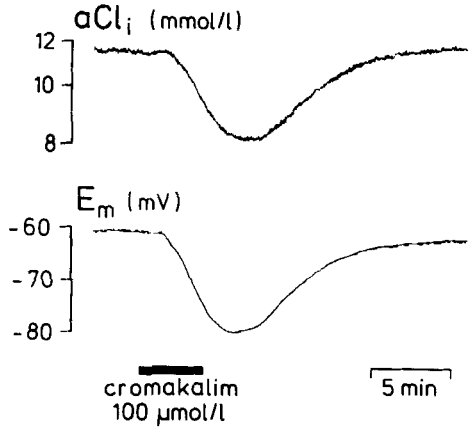

FIGURE 2. Effects of cromakalim on intracellular Cl^- activity (aCl_i) and membrane potential (E_m) of a muscle fiber from an isolated rat soleus muscle. Cromakalim was applied via the bathing solution during the time indicated by the bar.

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 $[K^+]_e$) a resting potential of −62 mV and an E_K of −95 mV was found. The membrane depolarized further and the difference between E_m and E_K increased when $[K^+]_e$ in the bathing solution was reduced to 1 mmol/L. (A membrane depolarization upon lowering of $[K^+]_e$ is a characteristic observation in isolated skeletal muscle fibers from patients with hypokalemic periodic paralysis.[14]) In this situation, the application of cromakalim resulted in a reversible membrane hyperpolarization of 35 mV. Later on, $[K^+]_e$ was raised to 3.5 mmol/L. This produced only a slight increase in E_m, whereas aK_i returned to its baseline level. Similar observations were made on another 4 fibers from 2 patients.

Table 1. Effects of cromakalim (100 μmol/L, application time 5 min) on membrane potential (E_m), intracellular K^+ activity (aK_i), and intracellular Cl^- activity (aCl_i) of fibers in rat soleus muscle and human muscle biopsies (control).*

Type of muscle	E_m (mV)	ΔE_m (mV)	aK_i (mmol/L)	ΔaK_i (mmol/L)
Rat, soleus (8;2)	−72.2 ± 5.7	−6.8 ± 1.7	88.9 ± 4.6	−2.7 ± 0.6
Human, control (12;2)	−73.3 ± 4.8	−10.7 ± 5.3	96.1 ± 6.7	−2.8 ± 1.1
			aCl_i (mmol/L)	ΔaCl_i (mmol/L)
Rat, soleus (11;3)	−71.0 ± 6.2	−12.2 ± 3.5	9.2 ± 2.1	−1.3 ± 0.8
Human, control (6;2)	−72.5 ± 7.3	−15.3 ± 10.4	8.0 ± 0.6	−2.0 ± 1.4

Mean values ± SD; the number of fibers and muscle preparations are given in parentheses.

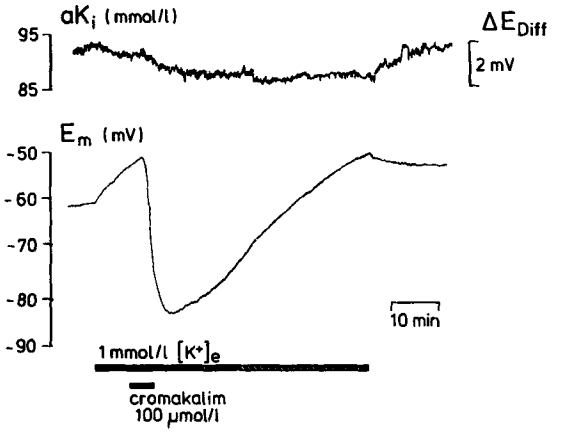

FIGURE 3. Effects of cromakalim on intracellular K^+ activity (aK_i) and membrane potential (E_m) of a muscle fiber in a skeletal muscle segment obtained from a patient with hypokalemic periodic paralysis. $[K^+]_e$ at the beginning of the recording was 3.5 mmol/L. It was reduced to 1 mmol/L to imitate the clinical situation in which low levels of $[K^+]_e$ are observed. E_{Diff} is the difference between the voltage reading of the K^+-sensitive and the reference barrel of the double-barrelled K^+-sensitive micro-electrode. Cromakalim was applied via the bathing solution during the time indicated by the bar.

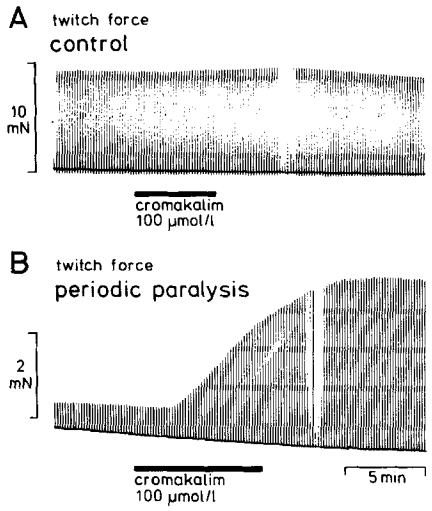

FIGURE 4. Effects of cromakalim on the twitch force of a skeletal muscle fiber bundle from a control person **(A)** and a patient suffering from hypokalemic periodic paralysis **(B).** The muscles were stimulated supramaximally every 10 sec with a voltage pulse (100 V; stimulus duration 2 msec). Cromakalim was added to the bathing solution (3.5 mmol/L $[K^+]_e$) during the times indicated by the bars. The effect of cromakalim on the twitch force of the diseased muscle was only slowly reversible (not illustrated, see text).

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[12,18] that tolbutamide antagonizes the effect of cromakalim on human skeletal muscle. Now we have found that glibenclamide, another sulfonylurea 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).

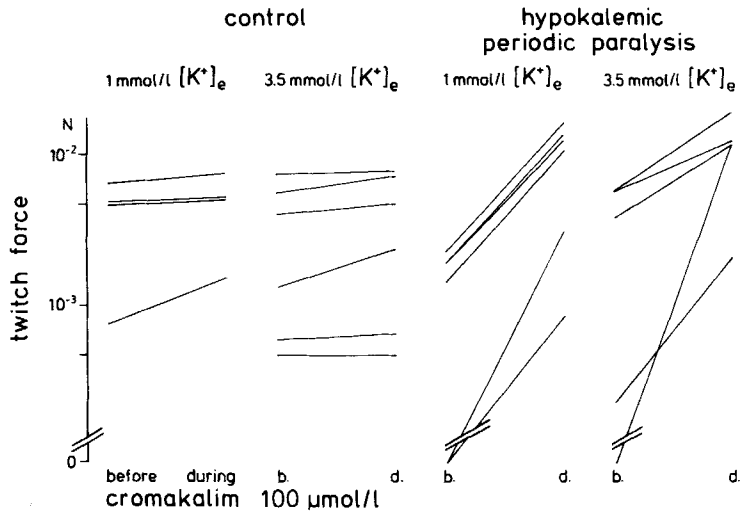

FIGURE 5. Summary of the effects of cromakalim on the twitch force of 12 skeletal muscle fiber bundles obtained from 6 control persons and from 4 patients with hypokalemic periodic paralysis. The bundles were stimulated every 10 sec as illustrated in Figure 4. The values of the twitch force, which were taken before and 5–10 min after application of cromakalim, are shown. Note the logarithmic scale.

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 8), in cardiac muscle cells[6,17] and in human skeletal muscle cells.[12,18] One of the consequences of such an effect should be an efflux of intracellular K^+ ions and a decrease in the intracellular K^+ activity.

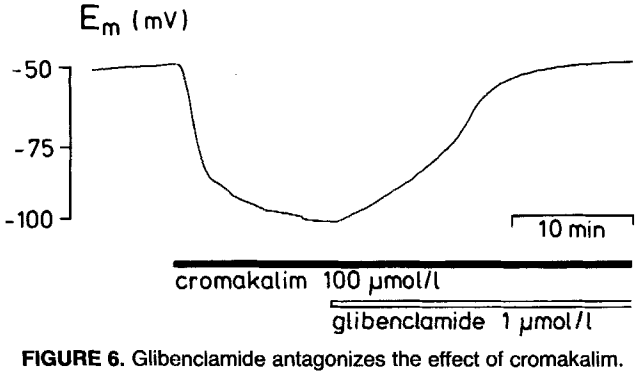

FIGURE 6. Glibenclamide antagonizes the effect of cromakalim. The figure shows a continuous recording of the membrane potential (E_m) of a skeletal muscle fiber from a patient with hypokalemic periodic paralysis. $[K^+]_e$ in the bathing solution was 1 mmol/L. Glibenclamide (1 µmol/L) was added to the cromakalim-containing solution.

In fact, the measurements with K^+-sensitive micro-electrodes in the present study revealed a diminution of aK_i. 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_i 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_i. Effects of cromakalim on the membrane Ca^{2+} conductance or Ca^{2+} 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^+ channels[7,19] and it antagonizes the effect of cromakalim in cardiac muscle[6,17] 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 A-channel, delayed rectifier, and calcium-activated potassium channels are not antagonized by glibenclamide.[4] 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.[16] This process is especially important because it operates around the resting membrane potential. Consequently, the observed repolarization could restore simultaneously normal excitability and contraction force. The second mechanism by which the cromakalim-induced repolarization could improve twitch force is based on the voltage dependency of the transduction protein between excitation and contraction.[9] Recently, this protein has been identified as the dihydropyridine receptor in the transverse tubule membrane. It functions both as the voltage sensor for excitation–contraction coupling and as the slow calcium channel.[20] The protein is half inactivated at a potential around −40 mV and can be reactivated by membrane repolarization.[2] Taken these results together, it is likely that the membrane repolarization induced by cromakalim (a) improves spread of excitation along the cell membrane and (b) enhances indirectly the release of calcium from the sarcoplasmic reticulum.

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.

Two main conclusions can be drawn from the present study. First, the data support the view[5,13,15] 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 therapeutical 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.

REFERENCES

1. Ballanyi K, Grafe P: Changes in intracellular ion activities induced by adrenaline in human and rat skeletal muscle. *Pflügers Arch* 1988;411:283–288.
2. Beam KG, Knudson CM: Calcium currents in embryonic and neonatal mammalian skeletal muscle. *J Gen Physiol* 1988;91:781–798.
3. Bretag AG: Synthetic interstitial fluid for isolated mammalian tissue. *Life Sci* 1969;8:319–329.
4. Cook NS: The pharmacology of potassium channels and their therapeutic potential. *TIPS* 1988;9:21–28.
5. Engel AG: Periodic paralysis, in Engel AG, Banker BQ (eds): *Myology.* New York, McGraw-Hill, 1986, pp 1843–1870.
6. Escande D, Thuringer D, Leguern S, Cavero I: The potassium channel opener cromakalim (BRL 34915) activates ATP-dependent K^+ channels in isolated cardiac myocytes. *Biochem Biophys Res Comm* 1988;154:620–625.
7. Fosset M, DeWeille JR, Green RD, Schmid-Antomarchi H, Lazdunski M: Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J Biol Chem* 1988;263:7933–7936.
8. Hamilton TC, Weston AH: Cromakalim, nicorandil and pinacidil: Novel drugs which open potassium channels in smooth muscle. *Gen Pharmacol* 1989;20:1–9.
9. Huang CLH: Intramembrane charge movements in skeletal muscle. *Physiol Rev* 1988;68:1197–1247.
10. Iaizzo PA, Lehmann-Horn F: Long cut muscle fibers repolarize and possess electrical and mechanical properties of intact human skeletal muscle. *Pflügers Arch* 1988;411:R191.
11. Lehmann-Horn F, Iaizzo PA: Resealed fiber segments for the study of the pathophysiology of human skeletal muscle. *Muscle Nerve* (to be published).
12. Quasthoff S, Spuler A, Lehmann-Horn F, Grafe P: Cromakalim, pinacidil, and RP 49356 activate a tolbutamide-sensitive K^+ conductance in human skeletal muscle fibres. *Pflügers Arch* 1989; 414:S179–S180.
13. Rüdel R: The pathophysiological basis of the myotonias the periodic paralyses, in Engel AG, Banker BQ, (eds): *Myology.* New York, McGraw-Hill, 1986, pp 1297–1311.

14. Rüdel R, Lehmann-Horn F, Ricker K, Küther G: Hypokalemic periodic paralysis: In vitro investigation of muscle fiber membrane parameters. *Muscle Nerve* 1984;7:110–120.
15. Ruff RL: Periodic Paralysis, in Seldin DW, Giebisch G, (eds): *The Regulation of Potassium Balance.* New York, Raven Press, 1989, pp 303–323.
16. Ruff RL, Simoncini L, Stühmer W: Slow sodium channel inactivation in mammalian muscle: A possible role in regulating excitability. *Muscle Nerve* 1988;11:502–510.
17. Sanguinetti MC, Scott AL, Zingaro GJ, Siegl PKS: BRL 34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle. *Proc Natl Acad Sci* (USA) 1988;85:8360–8364.
18. Spuler A, Lehmann-Horn F, Grafe P: Cromakalim (BRL 34915) restores in vitro the membrane potential of depolarized human skeletal muscle fibres. *Naunyn Schmiedeberg's Arch Pharmacol* 1989;339:327–331.
19. Sturgess NC, Ashford MLJ, Cook DL, Hales CN: The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 1985;8453:474–475.
20. Tanabe T, Beam KG, Powell JA, Numa S: Restoration of excitation–contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature* 1988;336:134–139.