To study the mechanism of attacks in familial hypokalemic paralysis, we recorded resting membrane potentials, action potentials, current–voltage relationships, and isometric forces in intercostal muscle fibers from three patients. In normal extracellular medium, the resting potential was reduced, but membrane conductance was not different from control. Excitability was reduced and the action potentials had no overshoot. On exposure to a 1-m*M* potassium solution, with or without insulin, the cells depolarized to about −50 mV, and became inexcitable. Over the tested membrane potential range from −120 to −40 mV, the slope conductance in the 1-m*M* potassium solution was not different from that of control fibers in a 1-m*M* potassium solution. In particular, the potassium component conductance was not reduced. Depolarized fibers could not be completely repolarized by returning to a 3.5-m*M* potassium solution. An experimentally induced transient shift of the chloride equilibrium potential to a highly negative value caused stable repolarization. Paralysis could also be induced by replacement of extracellular chloride with an impermanent anion, a treatment which causes myotonia in healthy fibers. It was concluded that the basic defects are a reduced excitability and an increased sodium conductance, and that these defects are aggravated on reduction of the extracellular potassium concentration.

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HYPOKALEMIC PERIODIC PARALYSIS: IN VITRO INVESTIGATION OF MUSCLE FIBER MEMBRANE PARAMETERS

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The familial disorder, hypokalemic periodic paralysis, is the longest known and most frequent form of periodic paralysis.[4,12] The contractile apparatus of the afflicted muscle fibers is normal[13] and the cause of paralysis was suggested to be inexcitability due to membrane depolarization.[39] The molecular events leading to depolarization are still mysterious.[30] An increase of the sodium conductance[22] or a reduction of the potassium conductance[23,35] have been proposed as the basic membrane defect, but it seems unlikely that a single abnormality of membrane function can account for all the known symptoms of the disorder. Recently, Layzer[26] pointed out that if the potassium conductance of the muscle fiber membrane were reduced, the electrogenic contribution of the Na/K-pump to the membrane potential would be greater than normal. Any possible reduction of the pump activity would then lead to an abnormally large muscle cell depolarization.

Several in vivo and in vitro muscle models have been investigated for behavior similar to that of muscles from hypokalemic patients, such as potassium-depleted[11,23] or barium-poisoned[16,41] muscle. But for a stringent test of the preceding hypotheses, it is necessary to measure component conductances of muscle cell membranes from patients. The results presented in this article were obtained in excitability tests, membrane voltage clamp experiments, and in the determination of the

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isometric force of excised intact intercostal muscle fibers obtained from three patients with familial hypokalemic paralysis. The experimental conditions were similar to those in earlier studies on paramyotonia congenita,[27] on adynamia episodica hereditaria,[28] and on control patients with no known muscle disease.[24,25] Some of the results have been presented to the Vth International Congress on Neuromuscular Diseases.[40]

CASE 1

A 56-year-old woman experienced her first nocturnal attack of muscular weakness at age 12. The attacks thereafter recurred three to four times per year, usually at night. After strenuous work or a carbohydrate-rich meal the weakness was sometimes so severe that she could hardly move her arms or legs. After two pregnancies, from age 25 onwards, the attacks were absent for several years. When they recurred at age 36, she saw a doctor who recorded a reduced serum potassium during an attack and diagnosed the disease as hypokalemic paralysis. She took potassium tablets for several years. Between age 40 and 45, the frequency of attacks decreased to zero. However, from then on the patient noticed a slowly increasing permanent weakness of the thighs. Both of her sons and a granddaughter experience attacks of weakness. Nothing has been reported about a similar affection of either her parents or her grandparents.

The musculature of the patient was well developed, except for slight thigh atrophy. The reflexes were preserved, but the strength of the hip and thigh muscles was markedly reduced. The patient could not climb stairs without the help of her hands. She could not stand up from a chair or lift her legs when lying on the back. The strength of all other muscles was normal. The electromyogram (EMG) of the biceps brachii was normal without myotonic discharges. Myopathic changes were detected at several sites of the quadriceps femoris. The serum potassium was 4.1–4.8 m*M*, the creatine kinase (CK) was 55–75 U/liter. The patient was euthyroid. She reported that from her early youth on she had experienced muscle weakness during cold weather independently from paralytic attacks. We, therefore, cooled her lower arm in a waterbath from 36 to 31°C.[18] This reduced the maximum force of fist closure considerably. No spontaneous activity was recorded in the EMG during cooling or during muscular work at the low temperature. The weakness was reversed 30 minutes after warming up.

A biopsy from the quadriceps femoris in 1978 showed diffuse atrophy, predominantly of type 2 fibers. A specimen of the latissimus dorsi muscle was taken during the present study. Light microscopy revealed numerous central nuclei and vacuoles of variable size. The fiber types were well differentiated with a slight predominance of type 1. Electron microscopy revealed dilatations of the sarcoplasmic reticulum, particularly near the Z-lines. The glycogen content of the fibers was normal.

Following the intercostal biopsy, the patient was treated for 3 months with a combination of prednisone, 50 mg/day, acetazolamide, 2 × 250 mg/day, spironolactone, 2 × 25 mg/day, and a low salt diet. This did not improve the permanent weakness.

CASE 2

A 33-year-old man had experienced episodic attacks of generalized weakness since age 12. At the beginning, the attacks occurred every 8 to 10 weeks, but by age 15, they occurred almost daily. The attacks usually began at night, lasting several hours. Sometimes he could not move his arms or legs for many hours. After such severe attacks full strength did not return for 2 to 3 days. No other member of the family is affected. The case has been thoroughly investigated and diagnosed as hypokalemic periodic paralysis.[31] Since age 29, treatment was acetazolamide, 4 × 250 mg/day, spironolactone, 4 × 25 mg/day, and a low salt diet, which resulted in an abolition of the attacks. After 4 attack-free years, a strenuous sporting event and a subsequent celebration with much sweet cake precipitated a very severe nocturnal attack of paralysis, which abated over the following day.

The patient's muscle bulk strength was normal. The EMG was normal without myotonic discharges. Serum potassium was 4.6 m*M*, CK was 96 U/liter. A muscle biopsy of the latissimus dorsi showed dilatations of the sarcoplasmic reticulum.

CASE 3

A 25-year-old man first noticed muscular weakness in his arms and legs at age 20. Since then, he often had spells of weakness, usually in the mornings. Most frequently, the force recovered within 1 to 2 hours, but sometimes he was unable to drive his car to work. After a full workout on a bicycle ergometer he was forced to sit down and could not stand up by himself for 4 to 5 hours. He reported that his father had had repeated incidents of muscular weakness when he was young. Also, his brother had experienced several episodes of slight weakness.

The patient had a well-developed musculature without atrophy. Interictal muscular force was normal. The EMG was normal without myotonic discharges. The interictal serum potassium was 3.8–4.6 m*M*, the CK was 75 U/liter. Following an oral dose of 160 mmole potassium, the serum potassium rose to 6.1 m*M*. The muscle strength remained unchanged. In another test, 1 liter of 20% glucose with 30 IU of insulin was given in a 60-minute intravenous infusion. The serum potassium decreased to 2.8 m*M*. At the end of the infusion period, considerable weakness of the proximal arm muscles was observed, and 15 minutes later the leg muscles were weak. Muscle strength redeveloped 2 to 3 hours later. Muscle specimens were taken from the biceps brachii, latissimus dorsi, and external intercostal muscles. Light microscopy revealed similar defects as reported for patient 1, most pronounced in the latissimus dorsi muscle.

Following the intercostal biopsy, the patient was treated with 4 × 250 mg acetazolamide for the first 4 weeks, then 2 × 250 mg/day. With this treatment, the patient did not observe muscle weakness even after strenuous exercise

METHODS

The patients gave informed consent for a muscle biopsy from the dorsal external intercostal muscle. The specimens were taken under general anesthesia without using muscle relaxants. Until we had gained some experience with these muscles, we stored the preparations in a Tetradotoxin (TTX)-containing Bretag's solution[3] with slightly elevated (4.5 m*M*) potassium content at 37°C to be sure to prevent depolarization. Later, when we knew how to establish high resting potentials in depolarized fibers (see the following), we stored the preparations in TTX-free 3.5 m*M* potassium solution at room temperature or at 8°C. Small muscle fiber bundles were dissected for the measurement of membrane potentials and for voltage clamp experiments as described earlier.[27] Depending on the fiber resting potential, voltage clamp step cycles were started from a holding potential of −80 mV, with steps increasing by 4 mV, first up to −120 mV, then down to −40 mV, or from a holding potential of −50 mV with steps increasing by 8 mV up to −130 mV. The data of the first two cases were processed using photographic records of the oscilloscope screen as described earlier.[27] With case 3, we used an improved data processing method. The signals from the voltage clamp set-up, membrane potential, V_1, potential difference, $V_1 - V_2$, and clamp current 1, were pulse-code-modulated and stored on a magnetic tape) Johne and Reilhofer, München, West Germany). Membrane length constants, fiber diameters, and current density-membrane potential relationships were calculated off-line from stored cycles using a PDP 11/03 computer (Digital Equipment Corporation, Maynard, MA). The computer averaged the amplitudes of the played-back signals at 3 times (40, 45, and 50 msec) after the beginning of each clamp step, calculated the current density for each membrane potential,[1] and plotted the relationships on an XY-plotter. The amplitudes of reliable data points were averaged to give the pooled characteristic curves shown in the Results section. The computer also calculated the slope of a straight line through the data points at the holding potential and 8 mV negative to the holding potential. This value approximates the specific membrane conductance at the resting potential. For depolarized fibers, the slope conductance at −80 mV was determined by eye from the pooled characteristic curves.

For a test of the excitability, fibers were impaled with 2 microelectrodes at a distance < 100 μm. One electrode was used for passing 60 msec-constant current pulses into the fiber, the other one for recording the resulting membrane potential changes.

The degree of paralysis was determined by recording the isometric force at optimal muscle length during tetanus contractions elicited directly by supramaximal electrical stimulation. Stimulation occurred with pulses of 0.2 msec duration in trains of 50 Hz and 200 msec duration at intervals of at least 5 minutes.

Solutions. Our standard solution was Bretag's[3] synthetic interstitial fluid having the following composition (in m*M*): NaCl 107.7, KCl 3.48, $CaCl_2$ 1.53, $MgSO_4$ 0.69, $NaHCO_3$ 26.2, NaH_2PO_4 1.67, sodium gluconate 9.64, glucose 5.5, sucrose 7.6. By gassing this solution with a mixture of 95% O_2 and 5% CO_2 the pH was set to 7.4. Solutions with 1, 4.5, and 7 m*M* potassium content were made by appropriate changes in the KCl content of the standard solution, without correction of the resulting osmotic changes. Chloride-free solutions were made by replacing NaCl and KCl with the respective methane sulfonate salts, and $CaCl_2$ by calcium gluconate. Tetrodotoxin (Roth, Karlsruhe, W. Germany), when added, was at 0.3 mg/liter, and insulin (Hoechst, Frankfurt/Main, W. Germany) was at 100 IU/liter. The temperature of the bath could be varied quickly between 37°C, standard temperature) and 27°C.

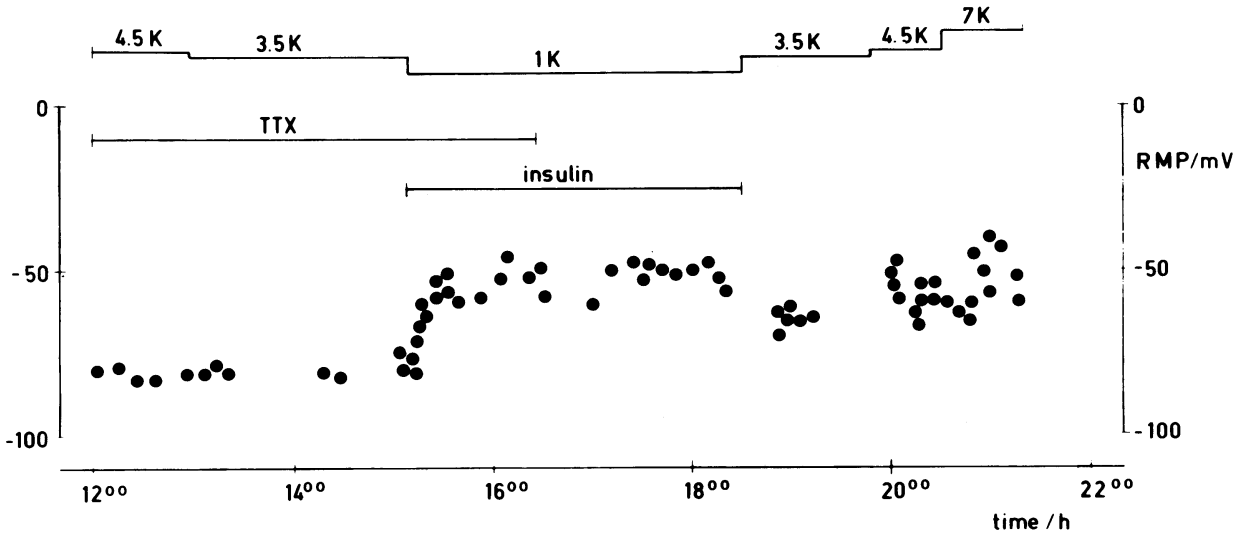

FIGURE 1. Resting membrane potentials recorded in fibers from patient 2. Measurements began shortly before mid-day, 3 hours after the surgical removal of the preparation. The preparation was first bathed in the 4.5-m*M* potassium solution containing tetrodotoxin (TTX). All fibers tested had resting potentials that were only slightly (5–10 mV) lower than those of healthy controls. The insulin-containing 1-m*M* potassium solution caused depolarization despite the presence of TTX. Application of solutions containing 3.5, 4.5, and 7 m*M* potassium did not re-establish high resting potentials.

RESULTS

Anesthesia and biopsy were without complications in all three patients. No abnormal twitching of the thoracic musculature was conspicuous, as had been the case with paramyotonic patients.[27] In contrast to the results from two patients with adynamia episodica,[28] the results from all three patients were highly consistent.

Membrane Resting Potentials and Excitability. As an example of the principal experiments carried out with bundles from each patient, Fig. 1 illustrates the various changes in resting potential recorded from many different fibers in a preparation from patient 2. At the beginning, the bundle was in a TTX-containing 4.5 m*M* potassium solution which was subsequently exchanged for a TTX-containing 3.5 m*M* potassium solution. The resting potentials were between −70 and −80 mV, i.e., slightly lower than the mean value of −83.3 mV observed in healthy controls.[24] After the challenge with a 1-m*M* potassium solution containing 100 IU/liter insulin, all fibers tested were depolarized to about −50 mV despite the presence of the sodium channel blocker TTX (Table 1). Healthy control fibers hyperpolarized in 1 m*M* potassium solution containing insulin (Table 1). Readmission of 3.5 m*M* potassium solution (without TTX) resulted in repolarization which did not reach the initial high value. An increase of the extracellular potassium concentration, $[K]_e$, to 4.5 m*M* and to 7 m*M* failed to re-establish stable high resting potentials (−58 and −56 mV, respectively). Similar results were obtained with bundles from patient 1.

In further tests with bundles from all three patients, we found that the depolarization on exposure to 1 m*M* potassium was independent of the presence of insulin (Table 1). In contrast, fibers from the control preparation hyperpolarized in insulin-free 1-m*M* potassium solution. TTX was not applied in these tests, and yet no spontaneous electrical or mechanical activity was observed during the depolarization. Direct electrical stimulation revealed that the bundles were paralyzed.

Since the fibers repolarized only incompletely on readmission of solutions with normal or increased potassium content, we tried to repolarize them by a transient shift of the chloride equilibrium potential to a highly negative value. The 1-m*M* potassium solution was first exchanged for a chloride-free solution containing 3.5 m*M* potassium, and after about 5 minutes of equilibration, this solution was exchanged for the chloride-containing, normal Bretag's solution. When tested a few seconds after the solution change, all fibers had stable resting potentials of the initial −70 to −80 mV. On electrical stimulation the fibers contracted; the depolarization thus proved to be easily reversible. This procedure was also of great value in these experiments because a paralyzed preparation could be transferred into the interictal state and used for another test.

Table 1. Resting potentials, membrane conductance values at −80 mV and fiber diameters recorded in three patients with hypokalemic periodic paralysis and a normal subject.*

Solutions	Patient 1	Patient 2	Patient 3	Normal subject V†
	Resting potentials (mV)			
3.5 m*M* K (Bretag's)	−71.2 ± 10.9 (48)	−79.2 ± 5.3 (29)	−77.1 ± 5.3 (29)	−85.5 ± 3.7 (29)
1.0 m*M* K	−55.5 ± 4.0 (16)	−51.3 ± 6.5 (35)	−56.3 ± 9.9 (33)	−95.5 ± 3.3 (13)
1.0 m*M* K + insulin	−53.5 ± 7.3 (35)	−52.3 ± 4.2 (51)	—	−99.4 ± 3.7 (17)
4.5 m*M* K	−71.2 ± 4.2 (31)	−80.0 ± 4.2 (5)	−74.7 ± 4.7 (53)	—
3.5 m*M* K, Cl-free	−42.4 ± 7.7 (37)	−51.2 ± 5.4 (11)	−47.2 ± 5.2 (28)	−82.6 ± 3.5 (10)
1.0 m*M* K, Cl-free	—	—	−41.6 ± 2.5 (9)	−54.2 ± 12.0 (20)
3.5 m*M* K, 27°C	—	—	−49.8 ± 5.5 (23)	−82.0 ± 5.5 (9)
	Slope conductances at −80 mV (μS/cm²)			
3.5 m*M* K (Bretag's)	130 ± 22 (9)	164 ± 24 (5)	174 ± 23 (10)	162 ± 27 (8)
1.0 m*M* K	110 (3)	107 (8)	105 (11)	97 ± 14 (6)
3.5 m*M* K, Cl-free	63 (8)	70 (2)	50 (10)	50 ± 13 (8)
1.0 m*M* K, Cl-free	—	—	84 (4)	98 (9)
	Calculated diameters (μm)			
Total mean	48.6 ± 8.5 (23)	60.3 ± 13.1 (24)	57.2 ± 6.0 (35)	50.3 ± 6.2 (31)

Mean values ± SD. The number of tests is given in parentheses.
†Control values from subjects I–IV are given in references.[26,27]

We also tried the same procedure at a maintained low $[K]_e$ by keeping depolarized fibers for 5 minutes in a chloride-free 1-m*M* potassium solution and then applying the chloride-containing 1-m*M* potassium solution. By this treatment we achieved only a transient repolarization. Several fibers attained a high resting potential in the 1-m*M* potassium solution long enough for us to record current–voltage relationships of the non-depolarized state. Within a few minutes, all fibers were again depolarized to −50 mV.

When hypokalemic fibers with high resting potentials were exposed to a chloride-free 3.5-m*M* potassium solution they depolarized to about −50 mV and remained depolarized (Table 1). No spontaneous activity was observed after the solution change. In contrast, control fibers transiently depolarized and went through a period of fibrillation after such treatment. Then they regained high resting potentials, were hyperexcitable and, on electrical stimulation, showed all signs of the myotonic reaction. We also tested the effect of a chloride-free 1-m*M* potassium solution in two bundles from the control subject. The majority of the fibers (16 out of 20 tested) had resting potentials below −50 mV. This illustrates that, even in healthy fibers, the combined destabilizing effects of low external chloride and potassium prevail over the hyperpolarizing effect of a highly negative potassium equilibrium potential.

In two bundles from patient 3 we investigated the effect of cooling on the resting potential. At 27°C, all fibers tested were depolarized to about −50 mV (Table 1), while control fibers had resting potentials of −80 mV. On rewarming, the fibers repolarized incompletely.

A consistent finding with fibers from all three patients under various conditions was a reduced excitability. In normal Bretag's solution, we were unable to elicit action potentials by intracellular application of depolarizing current pulses in fibers with resting potentials of less than −70 mV. When we raised the starting potential to −80 mV with hyperpolarizing prepulses, all fibers could generate action potentials but the spikes were without overshoot. In voltage clamp steps into the range between −68 and −40 mV, excitatory sodium current was elicited, but in contrast to the situation in healthy fibers, this did not trigger enough movement to dislodge the electrodes. In the 1-m*M* potassium solution, the fibers were usually depolarized to a potential value at which the excitatory sodium current would be inactivated in healthy fibers. We, therefore, repolarized the fibers from patients 1 and 2 using the voltage clamp. No excitatory current could be detected during clamp steps to −40 mV when the steps started from a holding potential of −80 mV. When the steps started from a holding potential of −110 mV, just an indication of an excitatory current could be detected. With fibers from patient 2, we also attempted to elicit action potentials by anodal break stimulation. Figure 2a illustrates the largest active response obtained in four tests. Current injection hyperpolarized the membrane from its resting potential of −55 mV to −140 mV. At the end of the hyperpolarizing pulse, the membrane potential returned to its resting value with

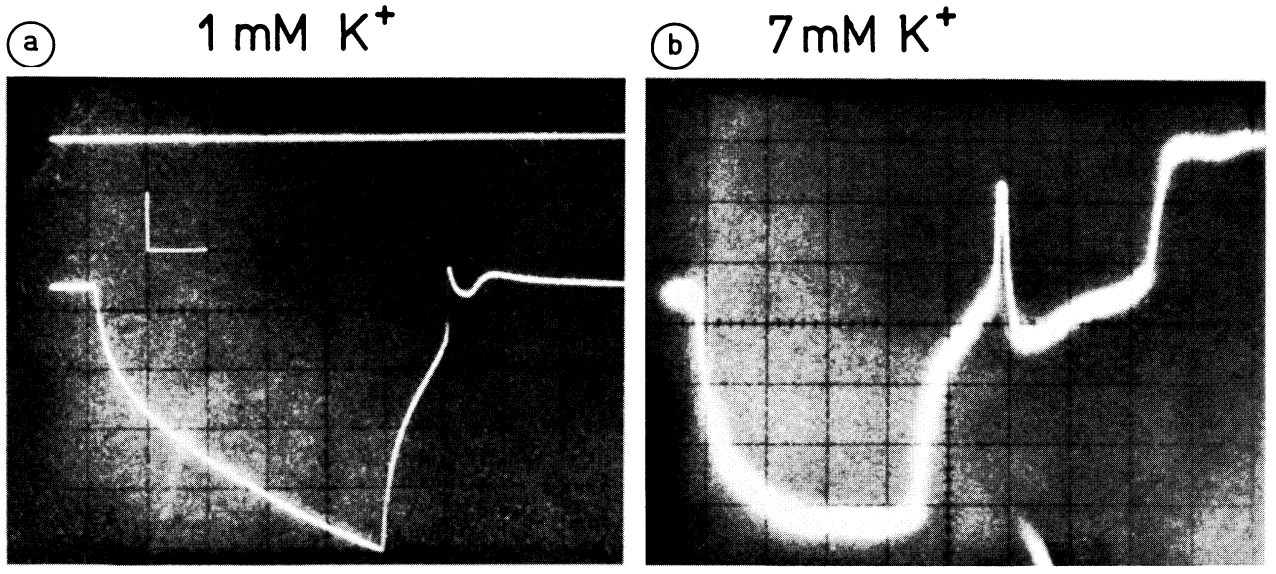

FIGURE 2. Anodal break stimulation of active responses in two fibers from patient 2. The potassium concentration of the bathing solutions **(a)** 1 m*M* and **(b)** 7 m*M* were so chosen that the fibers had about the same resting potential. Hyperpolarizing current was injected into the fibers to raise the membrane potential to about −140 mV. On the switch-off of the current pulse, the membrane potential returned to its resting value with a very small active response in the 1-m*M* potassium solution, and with an action potential in the 7-m*M* potassium solution. Note the different time constants of hyperpolarization caused by the differing membrane resistances. Calibration bars: time, 10 msec, potential, 20 mV. The upper trace in **(a)** indicates zero potential.

just a faint indication of an active response. Figure 2b illustrates a similar experiment with these fibers in a 7-m*M* potassium solution. In six tests, the active response was consistently larger although healthy fibers would have generated a spike with an overshoot. Since the resting potentials and the starting potentials for the anodal break excitation were about the same for the tests in the 1- and 7-m*M* potassium solutions, these results suggest that the excitability of hypokalemic fibers in low $[K]_e$ is more reduced than in high $[K]_e$.

Characteristic Curves. To test the hypothesis of an altered membrane conductivity, we determined the relationship between the steady state current density and the membrane potential. The slope of such characteristic curves represents the specific membrane conductance at the respective membrane potential. Figure 3 shows characteristic curves recorded in the various solutions used. In all four panels, each patient is represented by the same solid symbol. The open circles are data from a control person. Additional control curves have been published.[25,27,28] Each data point is the mean of experimental values determined in several fibers, the number of which is indicated in each panel. Figure 3a shows characteristic curves in normal Bretag's solution. Over the whole range of membrane potential investigated, there is not much difference between the results from the three patients and from the healthy control. The slopes of all four curves are lowest around the resting potential. The increase in slope on depolarization is a property of the chloride conductivity. The increase in slope on hyperpolarization is called anomalous rectification. It is believed to be a property of the potassium channels within the tubular system. If we assume that these characteristic curves represent the condition of the hypokalemic muscle fibers during the interictal state, this result shows that, between attacks, the total membrane conductivity is more or less normal.

Figure 3b shows the characteristic curves in a 1-m*M* potassium solution. In this solution, the fibers from the patients were depolarized while control fibers were hyperpolarized (Table 1). The dependence of the slope on the membrane potential between −120 mV and −40 mV was the same in all four curves. Comparison of characteristic curves recorded in the 3.5-m*M* and in the 1-m*M* potassium solutions (Fig. 3a and b) shows that anomalous rectification is much smaller in low $[K]_e$. The slope of the characteristic curves of hypokalemic fibers provides no evidence of a reduced g_K. We assume that the observed depolarization seen in hypokalemic fibers is caused by an increase in g_{Na}. As a consequence of the depolarization, the fibers gain chloride so that the chloride equilibrium po-

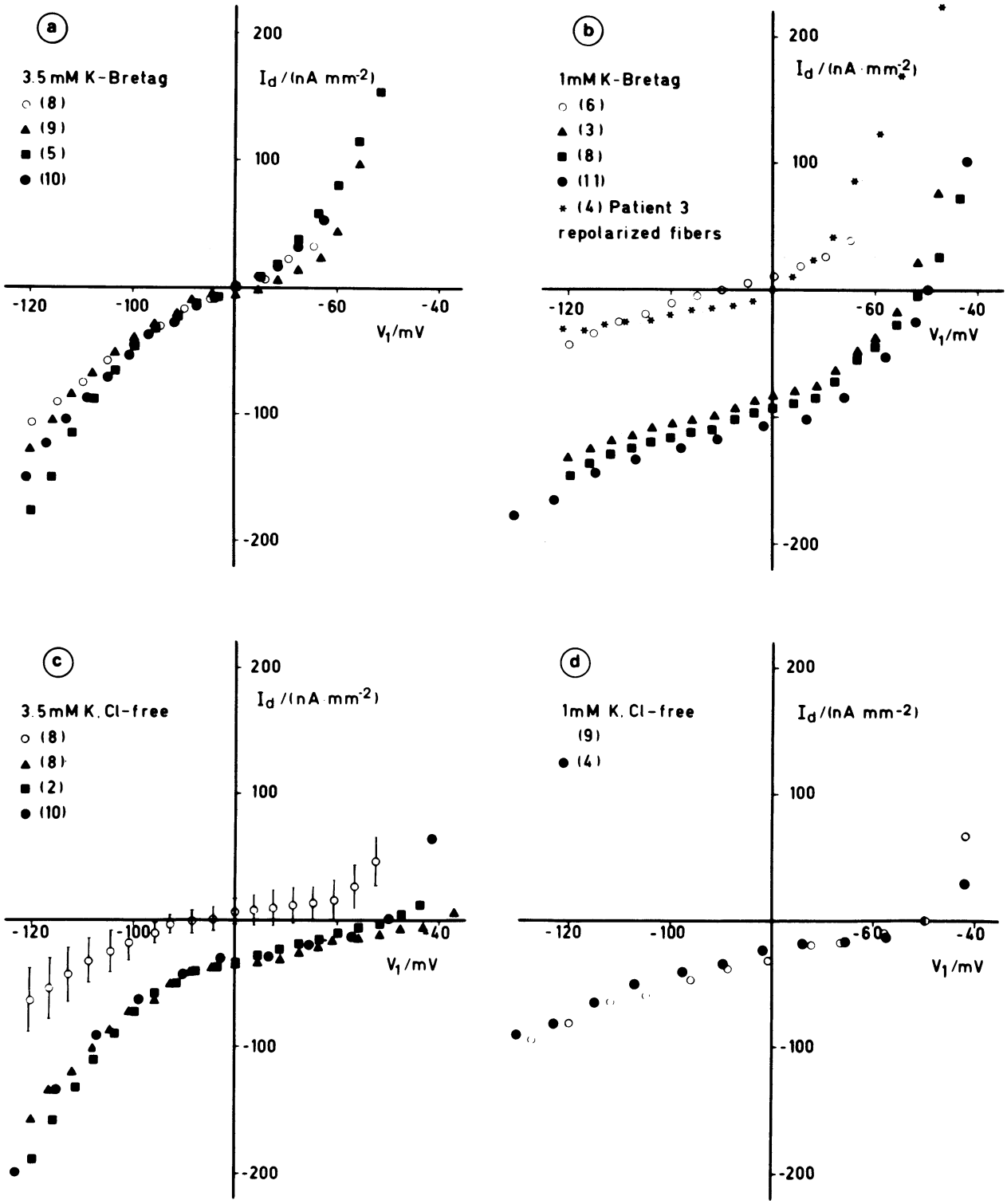

FIGURE 3. Steady-state current density—membrane potential relationships (characteristic curves) recorded in fibers from the three patients (▲, patient 1; ■, patient 2; ●, patient 3) and a healthy subject (○). **(a)** Shows characteristic curves in a normal extracellular medium (Bretag's solution containing 3.5 m*M* potassium). **(b)** Shows characteristic curves recorded in the 1-m*M* potassium solution. Asterisks indicate the curve obtained from patient 3's fibers after restoration of a high resting potential (see text). **(c** and **d)** Show characteristic curves recorded in chloride-free solutions containing 3.5 m*M*, **(c)** or 1 m*M* **(d)** potassium. All data points are averaged results obtained from the number of fibers indicated in each panel. In **(c),** typical standard deviations are indicated at the control curve. Note that in all solutions, the curves from patients and the control do not differ significantly in slope (membrane conductance).

tential is shifted to −50 mV. The most likely explanation for the observed downward shift of the characteristic curves of hypokalemic fibers is thus an increased inward current carried by sodium and chloride ions. In four fibers from patient 3, we were able to record the characteristic curves after the depolarized fibers had been repolarized by a 5-minute exposure to a chloride-free solution and readmission of extracellular chloride (see previous section). The characteristic curve was shifted upward along the current axis by the amount attributed to the chloride current, corroborating our previously made assumption.

Figure 3c shows characteristic curves of fibers in a chloride-free 3.5-m*M* potassium solution. Comparison of characteristic curves recorded in the chloride-containing and in the chloride-free 3.5-m*M* potassium solutions (Fig. 3a and c) shows that the overall conductivity is smaller in the chloride-free solution. In this solution, the fibers from the patients stayed depolarized while the control fibers regained a resting potential of −80 mV after a transient depolarization on admission of the chloride-free solution. Again, the slopes of all four curves had about the same dependence on the membrane potential between −120 mV and −40 mV. At −80 mV, the slope conductance of the diseased fibers was slightly larger than control (Table 1), indicating that g_K was not reduced. In chloride-free solutions, only sodium and potassium ions can carry current through the membrane. Assuming that the sodium current is nearly independent of the potential because the sodium equilibrium potential is far in the positive range, the characteristic curves approximate the potential dependence of the potassium conductivity. The most likely explanation for the downward shift of the characteristic curves of the hypokalemic fibers is an increased sodium current.

Figure 3d shows characteristic curves obtained from bundles from patient 3 and from the control subject in a chloride-free 1-m*M* potassium solution. In this solution, all the fibers from the patient and from the control were depolarized. The slopes of these curves are assumed to represent g_K during a severe attack. Over the whole potential range, the two curves do not significantly differ from each other, indicating that the g_K of the hypokalemic fibers is not abnormally reduced in low $[K]_e$. Note that, because of the depolarization, the resting g_K is larger than in normal $[K]_e$.

Conductance Values and Fiber Diameters. Table 1 contains the values of the slope conductances determined at the membrane potential of −80 mV. For fibers in normal Bretag's solution, this value represents the specific conductance of the resting membrane. No significant difference was found between the results from the three patients and the healthy control. For the low potassium and/or chloride-free solutions in which all hypokalemic fibers were depolarized, we have tabulated the mean slope conductances at −80 mV, because these values allow a more sensible comparison with the nondepolarized controls than the specific membrane conductances at the low resting potential. No significant difference was found between the respective results from the patients and the control.

Component conductance values can be calculated from the differences between slopes in chloride-containing and chloride-free solutions. At −80 mV, the averaged potassium and chloride component conductances, g_K and g_{Cl}, for the three patients were 61 and 95 $\mu S/cm^2$, respectively. Compared with component conductances of control fibers,[24] g_K is insignificantly larger and g_{Cl} is insignificantly smaller.

By using the same procedure, we also determined g_K at −80 mV in the chloride-free 1-m*M* potassium solution. The value obtained from four fibers of patient 3 was 84 $\mu S/cm^2$, 1.7 times larger than the value determined in the 3.5-m*M* potassium solution. Similarly, in control fibers bathed in the 1-m*M* potassium solution, g_K was about twice as large as in the normal potassium solution. These results are in conflict with the constant-field theory[20] which predicts a drop of g_K on reduction of $[K]_e$. A solution of this conflict is provided by the assumption of an increase of g_{Na} on reduction of $[K]_e$. This has been shown to exist in frog muscle fibers.[15,37]

The calculated diameters of fibers from the three patients and the control person were within the normal range[24,44] and were independent of the various extracellular solutions. We also measured the fiber diameters in histological sections of intercostal muscle bundles from patient 3. The mean value obtained from 800 fibers was 56.3 μm, in good agreement with the 57.2-μm calculated from the voltage clamp data (Table 1). In agreement with findings by Stern et al.,[44] the fibers from the two males (patients 2 and 3) had insignificantly larger diameters than those from the two females (patient 1 and control person).

Contraction Experiments. Lowering of $[K]_e$ to 1 m*M* consistently led to a reduction of the tetanic force

in hypokalemic bundles (to between 10% and 50% of the force in the 3.5-m*M* potassium solution), while the force of healthy bundles was unaffected by such treatment. When tested 20 minutes after readmission of the 3.5-m*M* potassium solution, the hypokalemic bundles had regained full force. As already mentioned in context with potential measurements, hypokalemic fibers were depolarized and inexcitable in the chloride-free solution, whereas controls showed hyperexcitability under this condition. The force of three bundles from patient 3 was <5%, and no sign of myotonic aftercontraction was noted. Ten minutes after readmission of the chloride-containing solution, the bundles had regained full force. Another condition that reversibly reduced tetanic force (to 75%) in hypokalemic bundles was cooling to 27°C.

DISCUSSION

The experiments carried out in a normal extracellular medium suggest that, in the interictal state, the muscle fibers of patients with hypokalemic periodic paralysis have slightly (5–15 mV) lower than normal resting membrane potentials and a reduced excitability. The resting potential seemed less stable than in healthy fibers, and this is probably the reason why some earlier investigators[21,22] recorded much lower values than we did. Resting potentials of the magnitude reported here have also been reported by others.[8,34,39,42,43]

Depolarization of the resting membrane to −50 mV and inexcitability were achieved in vitro by a mere lowering of the extracellular potassium concentration, $[K]_e$, a treatment that caused hyperpolarization without loss of force in the controls. This confirms the conclusion of early investigators of the disease[17,36,43] that the paralysis arises from a decreased $[K]_e$, and refutes the suggestion[21,22] that the drop in $[K]_e$ is only a circumstantial effect. It should be noted that the direction of potassium movement across the cell membranes is opposite during an attack and in our experimental low potassium situation, and yet in both cases the result is paralysis. This fact corroborates the notion that the reduction of $[K]_e$ is an important condition for the inexcitability. The relevant physiological effects of lowering $[K]_e$ are supposed to be a decrease of the steady-state potassium conductance, g_K,[19,24] and an increase of the steady-state sodium conductance, g_{Na}.[15,37]

To explain the pathologic depolarization, an abnormally low steady-state potassium component conductance, g_K, has been proposed as the basic defect in primary hypokalemic muscle fibers.[23,26,35] According to this view, g_K is just high enough to allow for a relatively high resting potential in the interictal state, and a reduction of the g_K/g_{Na} ratio, for instance, caused by lowering of $[K]_e$[19,24] or by insulin action[23] induces the critical shift of the membrane resting potential toward the sodium equilibrium potential. Another consequence of a reduced steady state g_K would be an increased contribution, E_p, of the Na/K-pump current to the resting potential, caused by the increased membrane resistance. This contribution has been guessed to be about 30 mV,[26] opposing depolarization in the interictal state. Since the Na/K-pump is inhibited by low $[K]_e$, the paralyzing depolarization has been suggested to be the consequence of the elimination of E_p during a supposed self-induced turn-off of the pump.[26] This hypothesis is not supported by our voltage clamp results. Our tests consistently failed to show an abnormally low specific membrane conductance (or g_K) in the normal and in the low potassium solution. Thus, there is no evidence suggesting a larger than normal contribution of the electrogenic pump to the resting potential in these muscles. Moreover, the drop of the serum potassium concentration to as low as 2 m*M* during a severe attack is unlikely to be sufficient for a self-induced block of the pump. For instance, in guinea pig ventricular muscle, the electrogenic pump current was found to be essentially unchanged when $[K]_e$ was varied between 2 and 4 m*M*.[9] We, therefore, suggest that the Na/K-pump is fully active during the whole attack and that its continuing elimination of potassium from the extracellular space is the reason for the persistence of the depolarization. The notion of an increased pump action preventing repolarization might seem paradoxical but it provides an explanation for the extremely slow recovery from hypokalemic attacks.

Our results favor the alternative hypothesis of an increased steady-state sodium conductance, g_{Na}.[22] Evidence for an increased g_{Na} is (1) the existence of increased intracellular sodium concentration between attacks,[22,33] (2) the in vitro result that removal of 90% of the sodium content from the external medium restored high resting potentials in depolarized hypokalemic fibers,[22] and (3) our confirmation of a reduced resting potential in normal $[K]_e$. Increased sodium current is also the most likely explanation for the observed differences between characteristic curves from hypokalemic and control fibers (Fig. 3b and c). We have calculated the increase in g_{Na} that is required to produce the observed downward shift of the characteristic

curves of hypokalemic fibers in the absence of extracellular chloride (Fig. 3c). The steady-state g_{Na} turned out to be increased by a factor of 3. This increased g_{Na} may be mediated by channels which are different from those which normally conduct the excitatory sodium current because they could not be blocked by the sodium channel blocker TTX. In agreement with this result, we found that the antiarrhythmic drug tocainide, which prevented paralysis in paramyotonia congenita,[38] did not have any beneficial effect in hypokalemic patients.

Increased steady-state g_{Na}, if present in the interictal state, must physiologically be met by an increased basal activity of the Na/K-pump in order to prevent too high a rise of $[Na]_i$. The instability of hypokalemic muscle fibers thus seems to arise from the narrow range of permissible activity of the Na/K-pump. If the pump activity is too low, depolarization may result from a rise in $[Na]_i$. If the pump activity is too high, depolarization may result from the lowering of $[K]_e$. Relative underactivity may be reflected in the often described weakness of hypokalemic patients in a cold environment,[29] expressed in vitro as a greater than normal drop of the tetanic force on cooling to 27°C. Overactivity may be related to paralysis as discussed previously.

In our experiments with low potassium or low chloride solutions, the process of depolarization was never associated with any spontaneous activity. This is in agreement with the clinical experience of absence of myotonia in patients with hypokalemic paralysis,[4] and it marks an important difference with respect to the mechanism of paralysis found in paramyotonia congenita[27] and in episodic adynamia when the latter is associated with myotonic runs in the interictal EMG.[29] While in these two diseases paralysis occurs because the fibers depolarize to a level at which the excitatory sodium current is normally inactivated, in hypokalemic fibers action potentials could not be elicited when the resting potential was as negative as -70 mV. This explains why in patients paralysis can occur even during a relatively small drop of $[K]_e$ accompanied by only slight depolarization (to about -70 mV).[33,42,43] In that respect, the mechanism of hypokalemic paralysis resembles that of episodic adynamia with complete absence of myotonic runs.[28] We do not know to what extent the two defects, an increased steady-state sodium component conductance and a reduced excitability, are linked in hypokalemic periodic paralysis.

Insulin was not a necessary factor for paralysis when $[K]_e$ was lowered experimentally. This is a surprising result because an altered influence of insulin on the metabolism of hypokalemic muscle fibers has been speculated upon.[21,22] In vivo, insulin certainly plays a prime role in provoking an attack. The hormone's crucial effect may consist in the well-known reduction of $[K]_e$[6,7,45] by way of its stimulating effects on the Na/K-pump. Insulin has been found to increase the relative sodium affinity of the transport mechanism[2,32] and to unmask latent cation pumping sites.[14] These effects do not involve glucose transport[6] or protein synthesis.[14] The latent sites are not available for ouabain binding or pump inhibition.[14] This may explain why even high doses of digoxin did not prevent paralysis induced by glucose and insulin.[10] In muscle fibers of hypokalemic patients, the interictal intracellular sodium concentration, $[Na]_i$, has been found to be increased by 10%[22,33] so that an increase by insulin of the sodium affinity of the Na/K-pump might be particularly effective. This may explain why in hypokalemic patients a certain test dose of insulin usually causes a greater and longer lasting fall in the plasma potassium concentration than in normals (unpublished results and reference[29]). As an additional effect, insulin reduces the potassium component conductance.[21] This increases the instability of the resting potential and might lead to the critical depolarization in vivo when $[K]_e$ is not as low as in our experimental situation. The attack-provoking effect of a carbohydrate-rich diet could be confined to the liberation of pancreatic insulin, and no further metabolic defect needs to be involved. Likewise, the attack-provoking effects of stress and of arterial infusion of adrenaline[12] can be explained by the stimulating effect of the catecholamines on the Na/K-pump.[5]

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