Membrane potentials, current-voltage relationships, and component con-
ductances were determined in resting excised external intercostal muscle
fibers from five patients with paramyotonia congenita. At 37°C all investigated
parameters were normal. At 27°C the resting potentials decreased to about
−40 mV, and the fibers were inexcitable. At this stage the membrane cur-
rents were much larger than in normal fibers owing to increases in the
membrane conductances for Na and Cl ions. The earlier finding that in the
cold the Na permeability is abnormally large was confirmed. The Cl perme-
ability was shown to be normal even in the cold. The decrease of the resting
potential and the changes in the current-voltage relationship at 27°C could
be prevented by the use of the Na channel blocker tetrodotoxin (TTX) or by
bathing the fibers in a Na-free solution. Our previous conclusion that the Cl
conductance at 27°C was also increased when TTX was present was not
confirmed. Exposure of a muscle bundle to 7 mmol/l potassium did not lead
to excessive depolarization and paralysis.

MEMBRANE DEFECTS IN PARAMYOTONIA CONGENITA (EULENBURG)

FRANK LEHMANN-HORN, MD, REINHARDT RÜDEL, PhD, and KENNETH RICKER, MD

In 1978 we decided to collaborate in studying the membrane defects of muscle cells from patients with nondystrophic hereditary muscle diseases. Our idea was that in these diseases the steady-state membrane conductances for certain ions might be altered and that we could define these alterations by applying the newly developed three-microelec-trode voltage clamp method[1] to biopsied intercostal muscle from such patients. Paramyotonia congen-ita was the first disease studied because we knew three patients who would consent to having a bi-opsy performed. At that time, very little was known about the pathomechanism of the characteristic

paramyotonic symptoms: muscle stiffness and weakness induced by exercise of the muscles in the cold.[2,5] The voltage-clamp experiments indeed showed that at 27°C the Na conductance of para-myotonic muscle fibers is abnormally high. This causes a long-lasting membrane depolarization that in turn leads to inexcitability of the muscle fibers. This inexcitability was suggested to be the reason for the paramyotonic weakness.[12] The param-yotonic stiffness also seems related to the abnormal Na conductance and the associated membrane de-polarization.[18] A decreased concentration of en-ergy-rich phosphates has been excluded as a reason for the stiffness.[10]

Since our first voltage-clamp study on muscles from paramyotonia patients appeared,[12] we have increased our experience with human intercostal muscle by studying biopsies from patients with adynamia episodica hereditaria[11,13] and hypoka-lemic periodic paralysis.[19] We have also collected many control measurements from patients without muscle disease.[7,8] At this state of the art, we were interested in reinvestigating our very first findings and in enlarging our knowledge of the membrane defect in paramyotonia congenita. Therefore, we continued our voltage-clamp studies with five fur-ther biopsies. This paper presents the new data in synopsis with the earlier findings,[12] so that our con-clusions are now based on the evidence obtained from six unrelated patients with paramyotonia con-

genita and from one patient with paralysis periodica paramyotonica.

PATIENTS

Patients 1 and 2 are the cases "PWOM A" and "PWOM B," respectively, of the first study.[12] New data were collected from patient 1 in a second biopsy. The data from patient 2 are based entirely on experiments with the first biopsy specimen. They were carefully reevaluated, and a necessary change of interpretation is given in the Results section. The membrane parameters of patients 3–6 are given here for the first time. Patients 3, 4, and 5 are identical with patients 2, 3, and 4, respectively, of the study on paramyotonic stiffness,[18] which contains their case histories. The case history of patient 6 has not been described previously. This 40-year-old man had been suffering from cold-induced muscle stiffness and weakness since early youth. Attacks of spontaneous weakness were unknown. His father and his brother were reported to have the same symptoms. The musculature of the patient was only moderately developed. Clinical myotonia was not present in a warm environment, but myotonic discharges could be detected in the EMG of all muscles investigated. During cooling of the lower arm a severe myotonia developed that gave way to muscle weakness. The serum creatine kinase was slightly increased (64 U/l). In a biopsy from the m. quadriceps femoris, type 1 and 2 fibers were hypertrophied, and occasionally atrophic type 2 fibers were seen.

Patient 7 is identical to case "PWM D" of earlier reports.[6,12] At the time these reports were written, we were not aware of this patient's episodic attacks of work load- and potassium load-induced weakness. In the meantime he was diagnosed as having paralysis periodica paramyotonica, and his symptoms and their differential treatment were reported.[16] His membrane parameters are included here again for comparison and for discussion in the light of his new diagnosis.

METHODS

The study was approved by the Ethics Commission of the Technical University of Munich and was carried out in abidance with the Helsinki convention. All patients gave informed consent for an external intercostal biopsy to be taken. The biopsy procedure and the electrophysiological methodology were as previously described.[12] Some muscle fiber bundles from the same biopsy material were used to study the mechanism of paramyotonic stiffness.[19]

All surgery was performed under general anesthesia, without the use of depolarizing muscle relaxants. We prefer general to local anesthesia because the patients experience no pain and are perfectly still during the operation. The better operating conditions result in a much higher yield of intact fiber bundles in the excised specimens. None of the patients experienced any problems with the operation procedure or the general anesthesia. As a rule, the whole biopsy procedure requires no more than a 2-day hospitalization of the patient.

In the operating room the patients were covered with warmed blankets because in the first patient the typical cold-induced symptoms had developed when he woke up. An irradiation heater was used to prevent the operative site from getting cold, since in the very first biopsy, carried out without this precaution, more than 70% of the fibers had had low resting potentials when investigated later at 37°C.[12] The excised specimens were immediately dropped into a thermos bottle containing oxygenated standard solution at 37°C (Bretag's synthetic interstitial fluid,[3] to which 0.3 mg/l tetrodotoxin was added). Transportation of the muscle specimen, its dissection into bundles, and its storage always occurred in this solution and at 37°C. The Na-free solution used in one experiment contained (in mmol/l): choline chloride 140, KCl 3.5, $CaCl_2$ 1.53, $MgSO_4$ 0.69, and glucose 5.5. The pH of this solution was set to 7.3 by the addition of 0.6 g/l Tris and titration with HCl. The experimental chamber, the voltage clamp setup, and the experimental protocol were as previously described,[12] but the data collection and reduction procedures were simplified by the application of a PDP 11/23 computer system.[19]

RESULTS

Resting Potentials. As in the earlier study,[12] at 37°C the muscles from all paramyotonia patients had normal resting potentials of about −82 mV (see Table 1). This value was the same in the presence or absence of the sodium channel blocker tetrodotoxin (TTX). In the previous study of fibers from patient 1 (patient "PWOM A"[12]), we had reported a two-peaked distribution of resting potential values, one peak being close to −80 mV, the other one close to −40 mV. We had concluded that the fibers with low resting potential had gone into the paralyzed state because of improper handling of the specimen. In the specimen obtained from this patient in a second biopsy, all fibers had high resting potentials, i.e., −76 ± 5.2 mV (n = 16). During surgery on patient 3, the operative site was not

Table 1. Resting potentials, E_m, and membrane conductances, g_m, of muscle fibers from six nonrelated paramyotonia patients and from a patient with paralysis periodica paramyotonica together with control values from the literature.[7,8]

Solution	Bretag, 37°C		Bretag, 27°C			Bretag + TTX, 27°C	
	E_m (mV)	g_m at -80 mV (μS/cm²)	E_m (mV)	g_m at -40 mV (μS/cm²)	g_m at -80 mV (μS/cm²)	E_m (mV)	g_m at -80 mV (μS/cm²)
Patient 1	-74.9 ± 8.4 (26) [-46.5 ± 7.7 (26)]	168 ± 89 (6)	-38.1 ± 5.4 (56) [-77.6 ± 7.9 (14)]	815 ± 151 (7)	256 ± 32 (7)	-76.4 ± 7.7 (24)†	123 ± 24 (9)†
Patient 2	-79.9 ± 7.0 (41)	142 ± 30 (12)	-36.5 ± 8.7 (18)*	881 ± 225 (11)*	391 ± 123 (11)*		
Patient 3	-81.0 ± 10.3 (35) [-40.9 ± 15.5 (118)]	158 ± 61 (7) [113 (4)]	43.1 ± 18.2 (125)				
Patient 4	-84.1 ± 4.7 (40)	182 ± 48 (9)	-40.8 ± 13.2 (22) [-76.3 ± 3.9 (17)]			-79.1 ± 5.1 (23)	140 ± 33 (10)
Patient 5	-86.9 ± 4.0 (12)	151 ± 17 (4)	-36.0 ± 6.1 (38) [-81.3 ± 6.4 (29)]	537 ± 85 (5)	324 ± 60 (5) [100 ± 16 (5)]	-79.2 ± 4.1 (6)	144 ± 63 (5)
Patient 6	-87.2 ± 9.3 (46)	164 ± 41 (21)	[-76.3 ± 8.7 (53)]		[158 ± 46 (15)]		
Means of paramyotonia (n = 6)	-82.3 (200)	160 (59)	-40.3 (259)	786 (23)	335 (23)	-77.9 (53)	136 (24)
Paralysis periodica paramyotonica (n = 1)	-80.3 ± 5.7 (57)	92 ± 15 (9)	-44.1 ± 8.5 (19)	1111 ± 135 (5)	513 ± 112 (5)	-70.7 ± 7.6 (25)	379 ± 91 (5)
Controls (n = 15)	-83.7 (112)	168 (112)	-79.0 (26)		142.6 (11)	-78.5 (28)	123.5 (15)

Values are mean ± SD, and the number of fibers is given in parentheses.
Measurements were performed in normal extracellular fluid at 37 and 27°C and in the presence of TTX at 27°C.
Values in square brackets were excluded from calculation of means. For significance of these values see text.
*21–22°C instead of 27°C.
†Stored in TTX-Bretag solution at 8°C overnight.

maintained at body temperature because of a defect of the radiation heater (see Methods). This was the only biopsy in which we again found such a two-peaked distribution of resting potentials. We have listed the fibers with low resting potentials separately in Table 1 (in brackets) and did not include them in the calculation of the mean resting potential of paramyotonic fibers at normal temperature.

Cooling the fibers to 27°C in most cases led to a slowly progressing membrane depolarization that ended at a membrane potential of about −40 mV, in agreement with our earlier findings. When the electrical threshold was reached, spontaneous electrical activity began, as described.[12] For the fibers from patients 1, 4, and 5 we found two-peaked distributions of the resting potential at 27°C, similar to that described above for 37°C. In fibers from patient 6, cooling alone did not lead to a substantial depolarization. We believe that the fibers with high resting potential at 27°C had not (yet) gone into the state of paralysis. Therefore, we listed them separately (in brackets) in Table 1 and did not include them in the calculation of the mean resting potential at 27°C. Finally we found that all muscle fibers from paramyotonia patients depolarize permanently to about −40 mV when a preparation is stimulated as well as cooled.

Once the fibers were depolarized to −40 mV in response to cold exposure, they did not repo-larize on rewarming, at least not within the next 4 hours. Addition of TTX to the bathing solution did not lead to repolarization (Fig. 1). This is somewhat different from the effect of TTX on fibers from patients wtih adynamia episodica hereditaria in which the excessive depolarization in a 7 mmol/l potassium solution was reversed by the addition of TTX.[11,13] Tocainide, an antiarrhythmic drug that effectively prevents the paramyotonic symptoms in patients,[17,21] did not cause repolarization of the fibers in the cold when we applied it in 0.5 mM concentration to a paralyzed bundle from patient 1.

When tetrodotoxin was added to the bathing solution before the cooling, the resting potential of the paramyotonic fibers did not decrease more than that of control muscles, in agreement with our earlier findings (see Table 1). Also, when the temperature was lowered while the preparations were bathed in a sodium-free solution, the fibers did not depolarize. The mean resting potentials at 27°C were then −79.3 mV in 8 fibers from patient 4 and −78.6 mV in 13 fibers from patient 5.

In paralyzed fibers from patients with hypokalemic periodic paralysis, repolarization and restoration of contractility was greatly accelerated when the chloride equilibrium potential was transiently shifted to a highly negative value.[18] This shift is accomplished by a two-step procedure. First, the muscle is bathed in a chloride-free solution for about
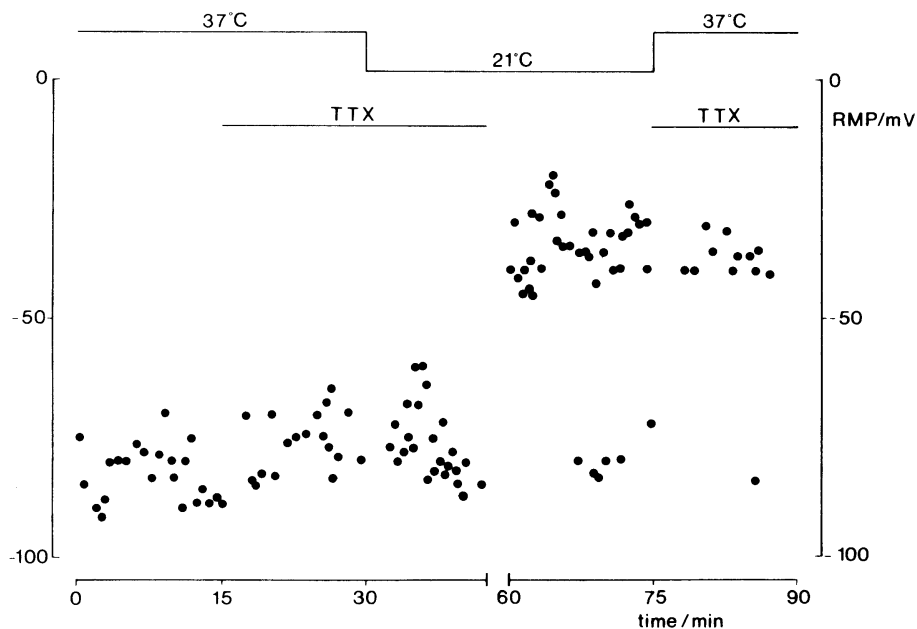

FIGURE 1. Resting membrane potentials recorded from a preparation from patient 2. At 37°C the potentials are high. On cooling to 21°C the potentials remain high as long as the Na channel blocker TTX is added to the bathing solution. On washout of TTX the potentials decrease to below −50 mV. Rewarming to 37°C and addition of TTX does not lead to repolarization.
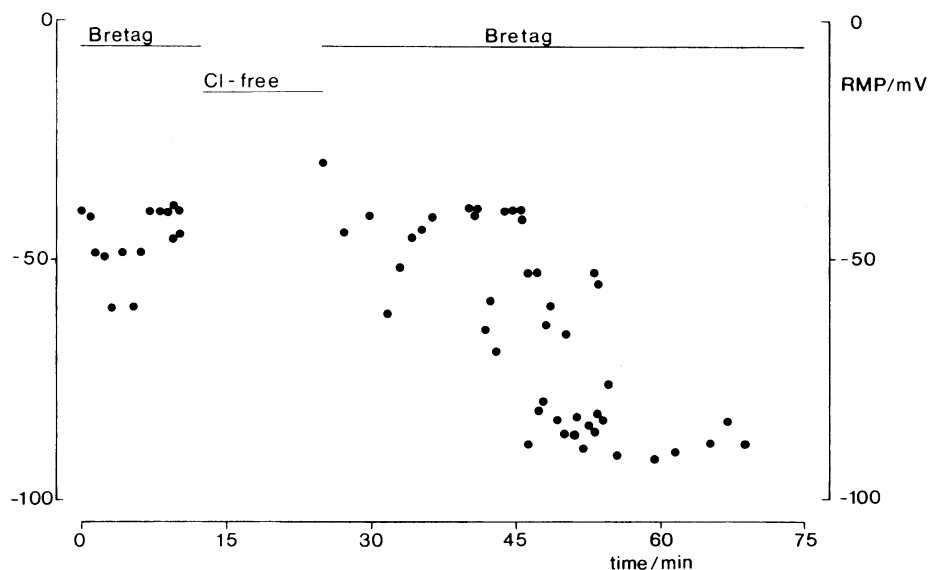
FIGURE 2. Resting membrane potentials recorded from a preparation from patient 3. At the beginning of these recordings the preparation had just gone through the solution and temperature changes illustrated in Fig. 1. The temperature was subsequently kept at 37°C. The TTX-containing standard solution was replaced by a TTX-containing Cl-free bathing solution for 15 minutes. No records were taken during this period. When the TTX-containing standard solution was readmitted, it took another 15 minutes before the resting potential increased substantially.

15 minutes, which leads to an almost complete loss of intracellular chloride; then, the muscle is bathed again in the normal chloride-containing solution, which leads to reuptake of chloride and repolarization. When we applied this procedure to a paralyzed preparation from patient 3, the mean resting potential increased from -46.7 mV (31 fibers) to -80.1 mV (24 fibers). It took the membranes more than 15 minutes in the TTX-containing standard solution at 37°C to attain a high resting potential (Fig. 2), whereas in hypokalemic periodic paralysis repolarization was complete immediately after readmission of the Cl-containing solution.[19]

Current-Voltage Relationships. The steady-state current-voltage relationships that we recorded in fibers from our new patients were very similar to our earlier results. We therefore decided to present the data as averages of all our experiments in Fig. 3. Since the number of successful tests was different for each patient, we did not draw error bars on the curves. The errors can, however, be estimated from the standard deviations of the slopes of the curves at -80 mV that are given separately for each patient in Tables 1 and 2 (g_m values). The results of the voltage-clamp studies may be summarized as follows. The current-voltage relationship of paramyotonic fibers at 37°C is not very different from control (Fig. 3A; the difference in the potential range negative to -100 mV is within the limits of

error of the two curves), but at 27°C it is completely different (Fig. 3B). The curve is shifted toward less negative potentials and is much steeper throughout than for normal fibers.

The Membrane Conductance at 27°C in the Presence of Tetrodotoxin. The most likely explanation for these differences between paramyotonic and normal fibers is the existence in paramyotonic fibers of an abnormal sodium conductance, g_m, that becomes manifest in the cold. In accordance with this hypothesis, the fibers did not depolarize much at 27°C when the sodium channels were blocked with TTX (Fig. 3C).

A surprising result of our first study[12] was that in 3 fibers from patient 2 "PWOM B") the membrane conductance increased even in the presence of TTX when the temperature was lowered from 37 to 27°C. Although the fibers depolarized by only about 3 mV, the slope of the current-voltage relationship was 2.8 times greater. In addition to these 3 fibers investigated on the day of the operation, we looked at 9 additional fibers after the preparation had been stored overnight at 8°C in TTX-containing Bretag's solution and warmed up the following day for a total of 12 fibers from this patient. In the latter 9 fibers the membrane conductance at 27°C was not increased when TTX was present.[12] In 1981 we had concluded that the 3 high-conductance values of the first-day measure-

A Bretag 37°C

● 6 patients
○ 8 controls

$I_d/(\mu A \cdot cm^{-2})$

20

10

-120   -100   -60 V_1/mV -40

-10

-20

B Bretag 27°C

● 4 patients
■ 3   "
○ 8 controls

$I_d/(\mu A \cdot cm^{-2})$

20

with TTX

10

-120   -100   -60 V_1/mV -40

-10

-20

-30

-40

-50

C Cl-free + TTX 27°C

● 3 patients
○ 4 controls

$I_d/(\mu A \cdot cm^{-2})$

5

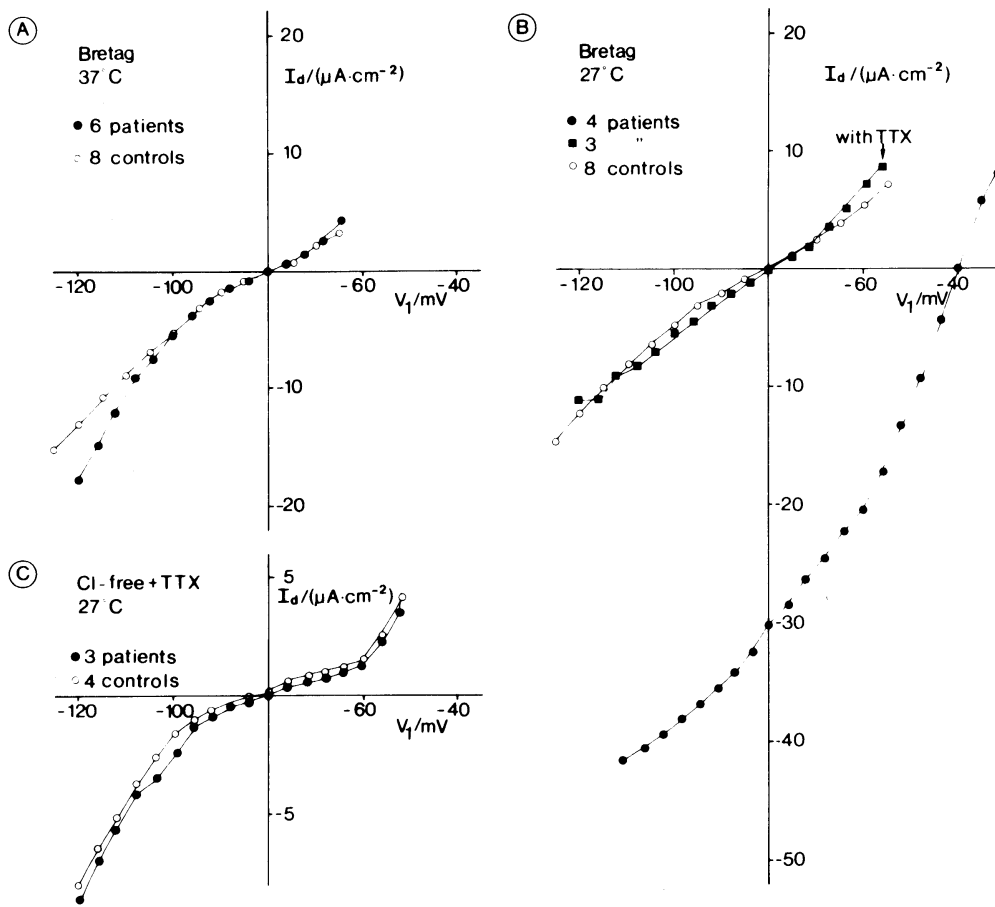-120   -100   -60 V_1/mV -40

-5

FIGURE 3. Current-voltage relationships recorded from intercostal muscle fibers biopsied from paramyotonia patients (solid symbols) and patients with no known muscle disease (open circles). The experimental points are averages of the results obtained from three to eight fibers from the number of patients given in each panel. Panel **A** shows that at normal body temperature the results from paramyotonia patients do not substantially differ from controls. Panel **B** shows that at 27°C the paramyotonic fibers are depolarized and have a much steeper current-voltage relationship (filled circles) than the controls (open circles), which hardly change upon cooling. When TTX is added to the bathing solution before the cooling, the paramyotonic fibers keep a normal current-voltage relationship (filled squares). Panel **C** shows that in the presence of TTX and in the absence of extracellular chloride, the current-voltage relationship of paramyotonic fibers at 27°C is not different from control, indicating that the potassium conductance of paramyotonic fibers is normal.

ment were more representative for paramyotonia than the 9 low values of the second-day measurement, because for patient 7 ("PWM D"), who at that time had not yet been diagnosed as having paralysis periodica paramyotonica (see above), the membrane conductance in the presence of TTX was also increased at 27°C.

We have now repeated this experiment testing 10 fibers from patient 4 and 5 fibers from patient 5 and found that g_m at 27°C was only insignificantly different from g_m at 37°C. In the light of the new evidence, we now believe that the low conductance value is representative. We have, therefore, entered this low value for patient 2 in Table 1 and have used it for the corresponding average value, which is also given in Table 2. Thus, in contrast to

our earlier paper,[12] we now state that in the presence of TTX the membrane conductance of paramyotonic fibers at 27°C is not abnormal. This statement is corroborated by results obtained from patient 7, whose fibers did not depolarize on cooling to 27°C, even in the absence of TTX. In all 15 fibers tested the membrane conductance was normal.

When a bundle is bathed in a TTX-containing, Cl-free solution, potassium is the only ionic species that can carry a substantial current through the membrane. The current-voltage relationship in this solution, therefore, represents the conductance of the potassium channels. As illustrated in Fig. 3C, this parameter is normal in paramyotonia congenita even at 27°C. It is remarkable that, as the de-

Table 2. Resting potentials, E_m, and membrane conductances, g_m, of muscle fibers from five nonrelated paramyotonia patients and from a patient with paralysis periodica paramyotonica together with control values from the literature.[7,8]

	Cl-free + TTX, 37°C		Cl-free, 27°C	Cl-free + TTX, 27°C	
	E_m (mV)	g_m at -80 mV (μS/cm^2)	E_m (mV)	E_m (mV)	g_m at -80 mV (μS/cm^2)
Patient 1			-36.5 ± 5.1 (6)		
Patient 2	-84.5 ± 4.6 (12)	41 ± 11 (8)	-37.4 ± 9.4 (4)	-84.2 ± 2.4 (10)	42 ± 9 (8)
Patient 3	-89.0 ± 4.0 (3)	72 ± 24 (3)			
Patient 4			-37.2 ± 7.8 (20)	-81.5 ± 3.2 (6)	50 ± 19 (5)
Patient 6	-85.8 ± 3.8 (5)	31 ± 6 (5)		-82.0 ± 3.6 (7)	46 ± 16 (8)
Means of paramyotonia ($n = 5$)	-85.5 (20)	44 (16)	-37.1 (30)	-82.9 (23)	46 (21)
Paralysis per paramyotonica ($n = 1$)	-80.6 ± 8.0 (10)	38 ± 5 (3)		-77.0 ± 6.4 (3)	47 ± 4 (3)
Controls ($n = 4$)	-82.6 (57)	42 (57)	-75.9 (21)	-74.4 (23)	41 (23)

Values are means ± SD, and the number of fibers is given in parentheses.
Measurements were performed in chloride-free extracellular fluid at 37 and 27°C and in the presence of TTX at 27°C.

polarization steps go beyond -60 mV, the potassium current increases more than one would expect from the constant-field equation. This suggests that in both normal and paramyotonic fibers the potassium permeability is rather large in the membrane potential range around -40mV.

The Chloride Conductance of Paramyotonic Fibers in the Depolarized State.

A comparison of the membrane conductance values determined at the clamped membrane potential of -80 mV in the presence and absence of extracellular chloride (g_m values in Tables 1 and 2) clearly shows that in the depolarized state the chloride conductance of the paramyotonic fibers is very large. Is the high value a simple consequence of the depolarization and the accompanying changes of the intracellular ionic concentrations, or is it also based on an increase of the chloride permeability, P_{Cl}? To answer this question we have used the nomograms of the Goldman equation given by Ruppersberg and Rüdel.[20] Figure 3 of this paper shows that, for a constant P_{Cl}, an increase of the chloride conductance (measured at $E_m = -80$ mV) by a factor of 2 is compatible with a decrease of the resting potential from -80 to -40 mV. The measured increase of g_{Cl} in the depolarized paramyotonic fibers is given by the quotient of g_m (Bretag) $- g_m$ (Cl-free) at 27 and 37°C (see Tables 1 and 2). Since this value, i.e., (335 $- 46$)/(160 $- 44$) $= 2.5$, is not much greater than expected for a constant P_{Cl}, the experiments indicate that the chloride permeability of the membrane of paramyotonic fibers is normal even when the fibers are in the depolarized state.

Effects of Increased Extracellular Potassium.

Cold-induced muscle weakness is not only observed in paramyotonia congenita but also in adynamia episodica hereditaria, the hyperkalemic form of periodic paralysis.[15] This has led to the speculation that paramyotonia and adynamia episodica might just be two facets of one nosological entity.[4,9] We have already shown for adynamia episodica that indeed in the excised muscle an abnormal decrease of the isometric force is not only observed when the potassium concentration of the bathing fluid is increased but also when the temperature is lowered to 27°C.[13] We have now performed the opposite experiment, testing the effect of a 7 mmol/l potassium solution on a fiber bundle from paramyotonia patient 4. The resting potential, measured in 15 fibers, was -71.1 ± 2.7 mV (mean $\pm$ SD). Normal muscle fibers had -69.0 ± 4.8 mV,[7] i.e., paramyotonic fibers did not excessively depolarize in the high-potassium solution. The membrane conductance, measured in four fibers at the resting potential, was not significantly different from control. The most important finding was that in contrast to fibers from adynamia episodica patients, the fibers from paramyotonia patient 5 produced full force when stimulated in the 7 mmol/l potassium solution. This finding marks an important difference between the two diseases.

Paralysis Periodica Paramyotonica.

Despite the result just described, there are families that clearly have the symptoms of both paramyotonia and adynamia episodica. As regards the membrane parameters, we have so far investigated only one pa-

tient with this combination called paralysis periodica paramyotonia. Basically, the membrane parameters had similar values as in "pure" paramyotonia. In particular, the characteristic increase in sodium permeability on cooling was observed in the fibers from this patient (Table 1). But one parameter, i.e., the absolute value of the chloride conductance and its temperature dependence, differed significantly from that of the rather homogenous group of six "pure" paramyotonia patients and of normal controls. At 37°C, g_m was too small, and at 27°C, g_m was too large. Thus, in addition to the sodium permeability, the chloride permeability also seemed abnormal in this patient. It should be noted that the chloride conductance was not abnormal in patients with "pure" adynamia episodica.[11,13]

DISCUSSION

The results of this study confirm the major result of our earlier report[12] that in paramyotonia congenita the Na channels show an abnormal behavior. Although at 37°C most of the Na channels seem to function properly, as judged by the normal upstroke and overshoot of the action potential,[12] in some of the channels the ability to go into the closed state after a physiological activation seems to be disturbed. Considering the fact that all patients have one or more paramyotonic symptoms even in the warm (lid lag, slowed muscle relaxation, spontaneous runs detectable in the EMG, percussion myotonia, long-lasting weakness after strenuous exercise),[18] we believe that the basic defect is present even at normal body temperature, although to a lesser degree than in the cold. Possibly at rest and during light work, an increased Na influx into the muscle fibers is met by an increased Na/K pump activity. This would explain why, despite some defective Na channels, the resting potential at 37°C is usually normal and why during experiments with excised muscles the resting potential sometimes was less stable than in normal muscles. Considering how often muscle weakness after strenuous work in the warmth is described in the case reports,[18] we believe that, during extended use of the muscles, more and more Na channels go into the noninactivating open state. This would cause an increased Na influx so that perhaps the Na/K pump could not immediately cope with such a load. In this case the resting potential would slowly decrease, and during this decrease electrical or even clinical myotonia could occur. Weakness would set in when the decrease of the resting potential was large enough for the

Na channels to become slowly inactivated. In the cold the transition of the Na channels into the defective state seems to be more probable. Since with lowering of the temperature the activity of the Na/K pump also is increasingly reduced, low temperature is exactly the condition when the paramyotonic symptoms should manifest themselves in their most distinct form. However, as we have noted with the resting potential of fibers from patients 1, 4, 5, and 6, sometimes low temperature alone is not enough to induce the paramyotonic depolarization. In agreement with the clinical experience, the addition of muscle activity then triggered the symptom. This suggests that the abnormal, noninactivating open state of the Na channel is preponderantly attained after a physiological opening.

We do not know whether all Na channels of a paramyotonic muscle fiber are capable of going into the abnormal open state, nor can we estimate the fraction of channels that are in this state when a muscle fiber is depolarized to approximately −40 mV, because nothing is known about the permeability of such an opened channel. The fraction is probably not too high because the ability to generate action potentials is immediately restored when a depolarized fiber is repolarized with the voltage clamp.[12] On the other hand this fraction seems higher than in other hereditary muscle diseases associated with muscle weakness since the degree of depolarization is so much larger than in paralyzed muscles from patients with adynamia episodica hereditaria ($E_R \sim -60$ mV)[11,13] or hypokalemic periodic paralysis ($E_R \sim -55$ mV).[19]

We have now managed to repolarize paramyotonic fibers after a cold-induced depolarization. The repolarization process proceeded remarkably slowly, but after about 30 minutes all fibers had assumed high resting potentials. This finding shows that the open Na channels may close again and do not have to be replaced by newly produced ones, as hypothesized earlier.[12] The low speed of repolarization after chloride withdrawal and readmission (Fig. 3) is difficult to explain. Why is it so much lower than in muscles from patients with hypokalemic periodic paralysis? Since recovery from depolarization means that the Na/K pump must remove the excess intracellular sodium that has accumulated within the muscle cells, one might think of the Na/K pump as another candidate for a defect in paramyotonia. Testing the hypothesis of a generalized defect in this disease, Marx et al.[14] investigated the temperature dependence of the ouabain-sensitive potassium uptake of red blood cells from patients 5 and 7. No abnormality was de-

tected. This result does not rule out the possibility that the Na/K pump of paramyotonic skeletal muscle is defective, but it also does not speak in favor of such a hypothesis.

The membrane conductance in the depolarized fibers was very large for all patients investigated. As we have pointed out in the Results section, this increase is a consequence of both the depolarization, since the potassium permeability is very large near -40 mV (see Fig. 3C), and of the altered distribution of chloride in the extra- and intracellular spaces associated with the depolarization. The latter causes an increased chloride conductance, although the chloride permeability of paramyotonic muscle is nearly unchanged at -40 mV. Since the Na/K pump produces an electric current, its repolarizing action depends on the membrane conductance. The higher the conductance, the lower the repolarizing effect of the pump. The time course of repolarization in Fig. 2 suggests that it takes the membrane a long time to repolarize from -40 to -60 mV, where the conductance is high, and that the speed increases as the conductance decreases.

A noninactivating sodium current has also been found to be responsible for the excessive depolarization of the muscle fiber membranes in adynamia episodica hereditaria.[11,13] One of the striking differences between the non-inactivating Na channels in paramyotonia and adynamia episodica is that in the latter case TTX quickly reverses the excessive depolarization, whereas in the former case TTX is ineffective once the membrane is depolarized. We do not know whether this is due to the larger depolarization in paramyotonic fibers.

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