Three patients with paramyotonia congenita and 3 control persons were biopsied for an in vitro investigation of the sarcolemmal membrane parameters and of the contractile properties of paramyotonic muscle. At 37°C, paramyotonic muscle fibers had normal resting potentials, but on cooling to 27°C they depolarized. Depolarization to −60 mV caused spontaneous activity, and further depolarization to −40 mV caused inexcitability. Depolarization could be prevented by the application of tetrodotoxin, a finding suggesting a defect in the Na channels. Analysis of the membrane current densities using voltage clamps with 3 microelectrodes revealed that in paramyotonic patients at 37°C all component conductances were normal, except for a decreased Cl conductance in the patient who had myotonia in a warm environment. At 27°C, the Na and Cl conductances were abnormally high. The K conductance was always normal. The results explain the clinical symptoms of weakness and paralysis. Potassium- and caffeine-contracture experiments gave normal results. The clinical symptom of paramyotonic stiffness, therefore, has not been explained by these studies.

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MEMBRANE DEFECTS IN PARAMYOTONIA CONGENITA WITH AND WITHOUT MYOTONIA IN A WARM ENVIRONMENT

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In spite of attempts to determine the muscle defect in paramyotonia congenita by using motor point biopsy of the forearm muscles,[8] the question remains whether the alteration lies in the cell surface membrane, in the intracellular membrane systems concerned with excitation-contraction coupling and relaxation, or in the contractile apparatus itself. A more detailed study of the pathologic mechanism in paramyotonia is needed for a successful management of the typical symptoms: myotonia, cold-induced muscle stiffness, and, in particular, weakness and paralysis. Such work may also give some clues to the physiological function of normal human skeletal muscle.

The present study was carried out with patients whose symptoms had been carefully defined.[7] Biopsy of external intercostal muscle was chosen on the basis of electromyographic findings, which showed that intercostal muscles, although being respiratory muscles, readily produce myotonic runs on movement of the needle.

The properties of the cell surface membrane were examined by a technique using voltage clamps with 3 microelectrodes. The following parameters of paramyotonic and normal muscles were evaluated: the resting membrane potential, the action potential, the specific membrane conductance, the component conductances, and the current density-voltage relationship. These parameters were measured at body temperature and at ≤ 27°C. The functional states of the contraction coupling and contractile structures were tested by using potassium and caffeine contractures.

The major finding of these tests is that the Na

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and Cl conductances of the cell membrane are abnormally sensitive to temperature. This has led to successful treatment of paramyotonic symptoms by application of the lidocaine derivative tocainide.[16] Some of the results have been reported to the German Physiological Society.[10]

METHODS

Three unrelated male patients gave informed consent to having a biopsy specimen taken from the dorsal external intercostal muscle. Two patients had been diagnosed clinically as having paramyotonia without myotonia in a warm environment (patients PWOM A and B), and 1 patient had paramyotonia with myotonia in a warm environment (patient PWM D). The clinical symptoms of these patients are described in detail in the accompanying report.[7]

The first specimen (from PWOM A) was taken under local anesthesia, i.e., during lidocaine blockade of the respective intercostal nerves. The specimens of the 2 other patients were taken under general anesthesia (halothane, barbiturate).

The excised specimens, measuring about 1.5 × 2.5 cm^2, were kept in gassed (95% O_2, 5% CO_2) standard bathing fluid (see below) at 37°C, which (except for patient PWOM A, see Results) contained tetrodotoxin (TTX, Roth, Karlsruhe, West Germany), 0.3 mg/liter, to suppress spontaneous activity. The specimens were dissected into 10 to 12 preparations containing 50 to 500 fibers each. In a similar way, normal muscle specimens were prepared from 3 patients who had thoracic surgery for reasons other than muscle disease. Some paramyotonic and normal preparations were embedded in araldite for histological examination and determination of fiber diameters.

The Voltage Clamp Set-Up. A preparation was placed in a 6-ml lucite experimental chamber which was continuously perfused with gassed bathing fluid at a rate of 3 ml/min. The temperature of the bath was controlled and could be varied between 38°C and 20°C. For contraction measurements, one tendon was hooked to a semiconductor strain gauge (Akers, Horton, Norway) connected to a chart recorder. Three microelectrodes (5–15 MΩ) were inserted into one of the fibers at about midlength for the determination of specific membrane parameters according to the method described by Adrian and Marshall[2] (electrode separation $l' = 200–350\ \mu m$, $l \simeq 2\ l'$; see Fig. 1A). The center and left electrodes were connected to high-input resistance amplifiers to record membrane potentials V_1 and V_2, respectively. The center potential V_1 was fed to a voltage-clamp amplifier which delivered clamp current to the right electrode. At full gain, a voltage-clamp step of 10 mV was 90% complete in less than 1 msec. The clamp current I was recorded in the bath return. The time course of the variables V_1, V_2, $V_1 - V_2$, and I during voltage-clamp steps lasting 40 msec was displayed on 2 oscilloscope screens (see Fig. 1B). At the same time the steady-state values ΔV and ΔI were plotted against ΔV_1 on a third oscilloscope operating in the XY-mode. A cycle of voltage-clamp steps starting from a holding potential of −80 mV and increasing in amplitude by 4 mV, first up to −120 mV, then down to −40 mV, was stored on the screen (see Fig. 1C) and photographed.

Determination of Membrane Parameters. A desk computer was programmed to calculate the longitudinal fiber resistance r_i (Ω/cm) and the length constant λ (μm) from the slopes of the $\Delta V/V_1$-curve and the $\Delta I/V_1$-curve at the resting potential. Using a myoplasmic resistivity, R_i, of 125 Ω·cm at 37°C[13] with a Q_{10} of 1/1.37,[9] the computer also calculated the fiber diameter, d_c, and the specific membrane conductance, g_m, at the resting potential. The specific membrane capacitance, C_m, was calculated from the time, τ_{84}, for V_1 to reach 84% of its final value in an unclamped square current pulse.[3] Membrane current densities were calculated from the following equation:[2]

$$I_m = 1/\pi d_c l\ [\Delta I/2 - \Delta V/r_i l]$$

and the current density–membrane potential relationships were plotted. For each muscle specimen, up to 20 fibers were investigated at 37°C and at low temperature (20°C–27°C). The results were pooled and standard deviations were calculated.

Determination of Contractile Properties. The diameter of the preparations used for contracture experiments was always less than 300 μm, permitting a fast equilibration of the applied agent in the extracellular space. One tendon was tied to the end of a Teflon rod dipping in a 20-ml vial filled with the desired solution, and the other tendon was connected to an isometric force transducer. The temperature of the bathing fluid was either that of the surroundings (23°C–25°C) or was kept at 37°C–39°C or at 11°C–12°C.

Na- and Cl-free solutions were used in the contracture experiments. To avoid action potentials, Na was replaced by Tris [tris(hydroxymethyl)aminomethane]. Propionate was used to

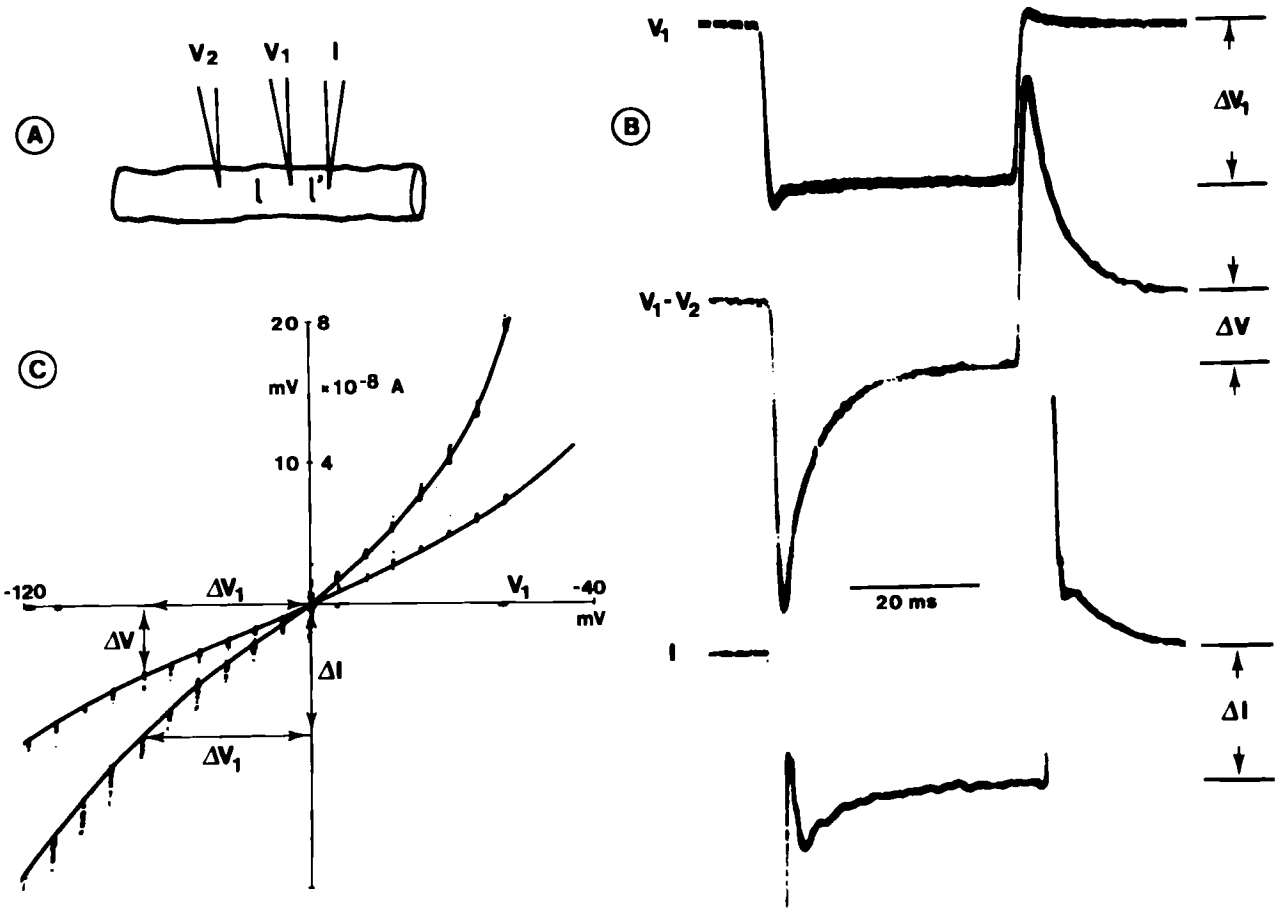

Figure 1. Examples of voltage clamp records taken to deduce specific membrane parameters. (A) Electrode arrangement. (B) Time course of the potential V_1, of the potential difference V_1-V_2, and of the clamp current I during a 24-mV hyperpolarization step ΔV_1, starting from a holding potential of −80 mV and lasting 40 msec. (C) Plot of the potential differences $\Delta V = \Delta V_1-\Delta V_2$ and the clamp currents ΔI against clamp potentials ΔV_1. Stepwise increase of clamp potential from a holding value of −80 mV (center of record) to −120 mV, then stepwise decrease from −80 to −40 mV. Each vertical bar reflects the change with the time of ΔV and ΔI, respectively, during the clamp step. Steady-state values are connected. Arrows indicate the −24-mV clamp-step values of record B which was taken at the same time. Normal subject I, 37°C.

avoid the shunting effect of the Cl ions reducing the level of depolarization caused by a given K concentration. The retarded repolarization upon reapplication of normal K was also prevented by the use of Cl-free solutions.

High K solutions were applied for as long as the developed force, having attained peak, decreased to one-half that value. At standard room temperature, this time amounted to about 10 sec when K was applied at a concentration of ≥ 100 mmol/liter.

The recovery of the contracture ability was tested as described previously.[14]

Solutions. The standard solution used for transportation, dissection, and basic experiments contained (in mmol/liter): NaCl 107.7; KCl 3.48; $CaCl_2$ 1.53; $MgSO_4$ 0.69; $NaHCO_3$ 26.2; NaH_2PO_4 1.67; Na gluconate 9.64; glucose 5.5; sucrose 7.6.[4]

The Cl-free solution used in voltage clamp experiments was made by replacing NaCl and KCl by the respective methane sulfonate salts, and $CaCl_2$ by Ca gluconate.

Tetrodotoxin (0.3 mg/liter) or tubocurarine (10 mg/liter) was added to these solutions in some experiments.

The control solution for contracture experiments contained (in mmol/liter): Tris 150, K 4, Ca 1.5, all as propionate salt. The high K solution contained: K 150 and Ca 1.5 as propionate. Solutions containing K at concentrations of 8, 20, 30, 50, and 100 mmol/liter were made by mixing appropriate amounts of these 2 solutions. In addition, a solution containing 400 instead of 150 mmol of K propionate/liter was used to determine maximum force.

Contractures were also produced by using Tris

Table 1. Resting potentials, specific membrane conductances at the respective resting potential, and temperature dependence ($Q_{10} = g_{37°C}/g_{27°C}$) of component conductances found in 2 patients who had paramyotonia without myotonia in a warm environment (PWOM A and B), in 1 patient who had paramyotonia with myotonia in a warm environment (PWM D), and in 3 nonparamyotonic controls.[a]

	Paramyotonic patients			Normal subjects		
Measurement	PWOM A	PWOM B	PWM D	I	II	III
Resting potential (mV)						
37°C	−72.9 ± 7.2 (10) −46.5 ± 7.7 (26)[b]	−79.9 ± 7.0 (41,	−80.3 ± 5.7 (57)	−83.2 ± 5.0 (6)	−82.1 ± 4.6 (24)	−83.7 ± 6.4 (16)
27°C	−36.6 ± 3.0 (20)	−36.5 ± 8.7 (18)[c]	−44.1 ± 8.5 (19)	−80.2 ± 3.5 (6)	−77.9 ± 2.6 (10)	−79.4 ± 2.7 (11)
27°C + TTX	—	−76.4 ± 7.7 (24)	−70.7 ± 7.6 (25)	—	−78.7 ± 4.6 (14)	−79.9 ± 4.7 (10)
Membrane conductance ($\mu S/cm^2$)						
37°C + TTX[d]	—	142 ± 30 (12)	92 ± 15 (9)	140 ± 28 (6)	165 ± 46 (9)	126 ± 32 (8)
27°C	263 ± 35 (5)[e] (633 ± 119)	391 ± 123 (11)[e] (881 ± 225)	513 ± 112 (5)[e] (1,111 ± 135)	—	147 ± 17 (5)	139 ± 29 (6)
27°C + TTX	—	398 ± 49 (3)[c]	379 ± 91 (5)	—	112 ± 27 (6)	159 ± 45 (4)
Cl-free, 37°C + TTX	—	41 ± 11 (8)	38 ± 5 (3)	—	32 ± 8 (5)	49 ± 14 (8)
Cl-free, 27°C + TTX	—	42 ± 9 (8)[c]	47 ± 4 (3)	—	30 ± 3 (6)	40 ± 11 (7)
Q_{10}						
g_K	—	1.00	0.85	—	1.05	1.15
g_{Cl}	—	0.25	0.15	—	1.25	0.80
g_{Na}	—	~0.15[f]	~0.15[f]	—	~1[f]	~1[f]
g_m	—	0.35	0.20	—	1.25	0.90

[a] *Data represent mean values ± SD (number of fibers evaluated). TTX = tetrodotoxin.*
[b] *Two populations of resting potential values were found in patient PWOM A, see text.*
[c] *Measured at 21°C.*
[d] *The conductance values in the presence and absence of TTX were not significantly different.*
[e] *Conductance values at the holding potential of −80 mV. Values measured at the low resting potentials are given in parentheses.*
[f] *Guessed from the amount of depolarization at 27°C.*

propionate solutions containing 2–50 mmol of caffeine/liter.

The control solution for acetylcholine contractures contained (in mmol/liter): sucrose 300, propionate 2, Ca 1. To this solution, 20 mg of acetylcholine/liter was added to give a concentration of 0.11 mmol/liter.

RESULTS

Anesthesia and biopsy were without complications in all 3 patients. Twitching of the thoracic musculature was conspicuous, particularly in patient PWM D.

For the first patient (PWOM A), the dissection of the muscle specimen was carried out in a TTX-free solution. Because of the careful temperature control, most of the muscle fibers were quiescent until the end of dissection. However, transport of the small preparations from the dissecting dish to the experimental chambers induced repetitive activity and twitching. To avoid it, TTX-containing solutions were used during dissection and transport to the experimental chamber in the remaining 2 experiments.

Resting Potentials and Spontaneous Activity. Fibers which had not been exposed to low temperature had resting potentials of −60 to −88 mV at 37°C, regardless of whether they were kept in TTX-containing or TTX-free solution. The results obtained with muscles from the 3 paramyotonic patients and 3 normal persons are presented in Table 1. There was no significant difference between the results from paramyotonic patients and normal persons with the exception of patient PWOM A whose preparation had shown spontaneous activity. In his case we found 2 populations of fibers, one having resting potentials at about −75 mV, and the other one at about −45 mV.

On cooling in TTX-free solution, the fibers depolarized slowly. Spontaneous electrical activity began when the membrane potential had fallen to about −60 mV. This occurred just below 30°C in most of the fibers from patients PWOM A and PWM D, and at about 20°C in patient PWOM B. The series of action potentials lasted for several minutes (i.e., much longer than usual myotonic runs) with a rather constant frequency of 1–5 Hz which only increased during the last seconds of the

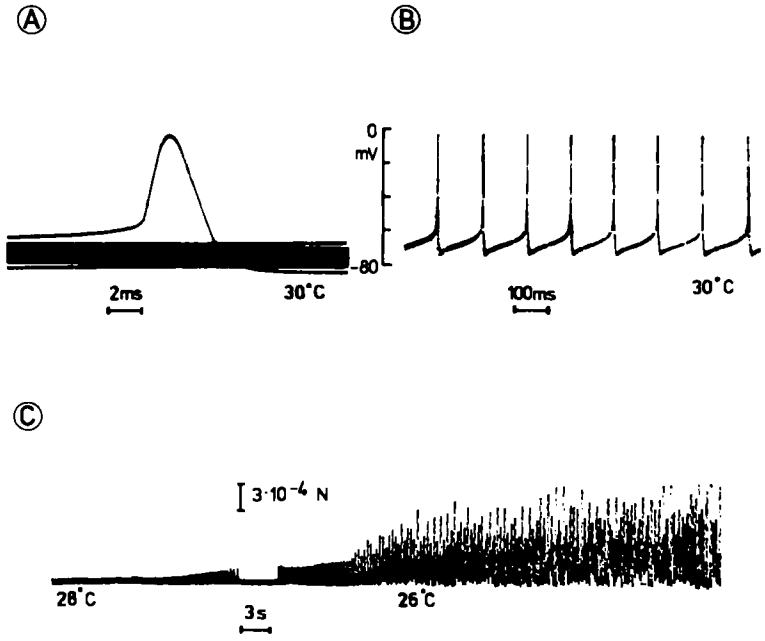

Figure 2. Spontaneous electrical (A,B) and mechanical (C) activity in a preparation from patient PWOM A. Record A shows superposition of about 10 successive sweeps of intracellularly recorded membrane potential. The fiber gradually depolarizes until near −60 mV when an action potential is set off. Note repolarization beyond −80 mV. Record B shows a series of action potentials recorded with slower time base after record A. Record C shows development of twitching during cooling of a small fiber bundle.

series. The changes in intracellular potential during one cycle from such a series are shown in Figure 2A. The recording was done with a freely running oscilloscope beam so that about 10 successive traces are superimposed. Depolarization began from about −80 mV, its speed slowly increasing to end in a fast spike. During repolarization, the potential reached −80 mV, suggesting that the K equilibrium potential and the fast K channel were normal. The cycle duration was about 200 msec. Slow time-base recordings of action potentials obtained from the same fiber 1 minute later are shown in Figure 2B. Tubocurarine (10 mg/liter) did not prevent the paramyotonic activity. A neurogenic origin of this activity can thus be excluded.

Spontaneous activity could also be detected by force measurement. Cold-induced twitches of a small fiber bundle from patient PWOM A are shown in Figure 2C. At 28°C, a single fiber twitched for about 10 seconds. At 26°C, several fibers joined in. Twitching was observed for 3 more minutes of cooling. It stopped at 20°C.

Investigation of different bundles from paramyotonic patients showed that nearly all fibers had depolarized to about −40 mV after cooling in TTX-free solution (see Table 1). Recovery to high resting potential was never observed, not even 20 minutes after rewarming to 37°C. Comparable depolarization and spontaneous activity was never observed in normal muscle fibers under the same conditions.

Depolarization, spontaneous activity and twitching were entirely prevented when the paramyotonic preparations were cooled in TTX-containing solution. The resting potentials of paramyotonic fibers were high at 27°C and not statistically different from those of normal fibers (see Table 1).

In summary, paramyotonic fibers have normal resting potentials at normal temperature, but on cooling they depolarize and start firing. They keep a high resting potential when a Na current blocker is present during cooling. This suggests that in paramyotonia the Na-carrying system is disturbed. The possibility cannot be excluded, however, that the component conductances for other ions are also altered. For example, a decreased K conductance (g_K) could induce depolarization, and a decreased Cl conductance (g_{Cl}) could cause repetitive activity similar to that in myotonia congenita. The following experiments were designed to clear these points.

Steady-State Current Density-Membrane Potential Relationships (Membrane Characteristics). Component Conductances. The membrane conductance of a muscle fiber is given by the slope of the membrane characteristic at the resting potential. Owing to the potential dependence of the 2 major component conductances, g_{Cl} and g_K, the slope of the membrane characteristic changes with the clamp potential. An investigation of the potential dependence of the slope of the membrane characteristic therefore gives clues to g_{Cl} and g_K.

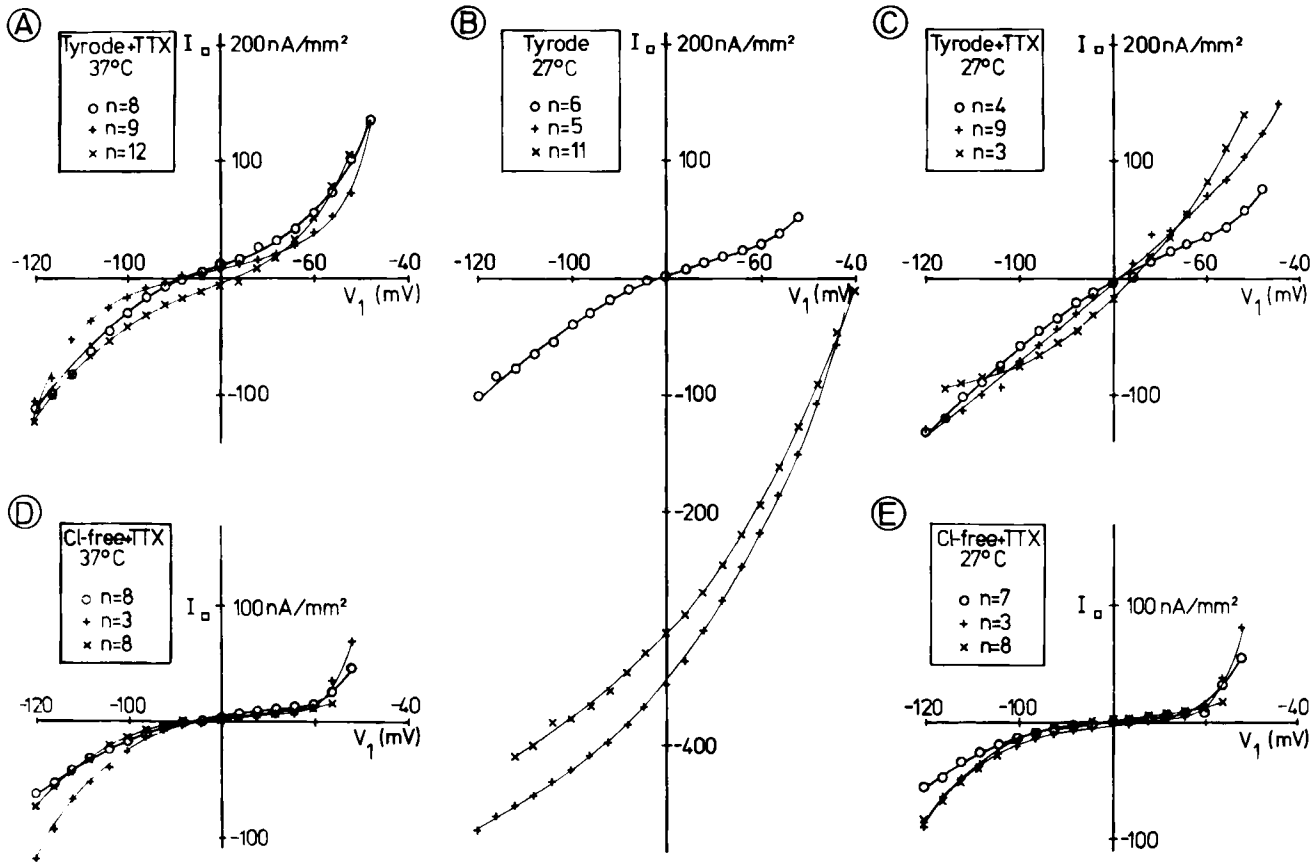

Figure 3. Current density–membrane potential relationships of intercostal muscle fibers from normal subject III (○) and from 2 paramyotonic patients PWOM B (x) and PWM D (+). For specification of solution, temperature of bath, and number of fibers investigated, see insets. Typical standard deviations are shown in Table 1. TTX = tetrodotoxin.

Figure 3A shows the membrane characteristics of normal and paramyotonic muscle fibers in TTX-containing solution at 37°C. Each data point represents the mean of the current density (positive current = outflow of cations) measured in 8 to 12 fibers. In both normal and paramyotonic fibers, the slope conductance is lowest at the resting potential. The increase in slope conductance on depolarization is called rectification, generally assumed to be a property of the Cl channel and, at stronger depolarization, also of the K channel.[1] The increase on hyperpolarization (anomalous rectification) is believed to be a property of the K channel within the tubular system.[1]

Comparison of the 3 membrane characteristics shows that—as far as slopes are concerned—the results from patient PWOM B are not much different from the normal membrane characteristic, suggesting that his K and Cl channels are intact under normal conditions. The membrane characteristic of patient PWM D is less steep over a large potential range, indicating a reduced membrane conductance. In particular, the resting membrane conductance was significantly ($P<0.01$) lower than normal in this patient (see Table 1). Similar results were obtained when TTX was omitted from the bathing solution.

If only g_{Cl} was reduced in patient PWM D, determination of membrane characteristics in Cl-free solution should have given similar results for this patient and for the normal subjects. In Cl-free solution all muscle fibers are hyperexcitable. To obtain stable conditions for a 3-microelectrode voltage clamp, we added TTX to the bathing solution. Figure 3D and Table 1 show that, indeed, there were no significant differences between the results in patient PWM D and those in the normal subjects (including patient PWOM B) in Cl-free, TTX-containing solution. The reduced resting membrane conductance found for patient PWM D at 37°C was therefore caused by a reduced g_{Cl}.

Since the paramyotonic symptoms are prominent in a cold environment, a more marked difference between normal and paramyotonic mem-

brane characteristics was to be expected at 27°C. For the experiments illustrated in Figure 3B, only muscles were used which had not been in Cl-free solution and which had been in TTX-free solution for at least 30 minutes. The membrane characteristics of paramyotonic fibers were found to be shifted to less negative potentials, to be steeper, and to have decreasing slopes on hyperpolarization. The potential shift corresponded to the depolarization produced on cooling, as already described. The increased steepness could be due to an increased g_{Cl}, g_{Na}, or even g_K. The decreasing slope on hyperpolarization could be due to an increased g_{Cl} or to a decrease of the tubular K conductance.

Table 1 gives the membrane conductance values measured at 27°C. Because of the great difference in resting potentials (40 mV), a comparison of conductance values of paramyotonic and normal fibers is of restricted value. For the depolarized paramyotonic fibers, we therefore determined the slope conductance at the holding potential of −80 mV also. The values are given in Table 1. For all 3 patients they were much higher than at 37°C.

The most likely explanation for this is a cold-induced increase of g_{Cl}. This hypothesis was tested by measuring the membrane characteristic in Cl-free solution. Since TTX had to be added to the Cl-free bathing solution, we first had to determine the control characteristic in TTX-containing standard solution at 27°C. The results are illustrated in Figure 3C. The normal membrane characteristic was not significantly different from that at 37°C, but the paramyotonic characteristics were steeper and showed a decreasing slope on hyperpolarization (very distinct for patient PWOM B, faint for patient PWM D). The shift to less negative potentials was absent in the presence of TTX (in agreement with the normal resting potential, Table 1), which makes it possible to compare the resting conductances at 37°C and 27°C in the presence of TTX (Table 1). The membrane conductance of paramyotonic muscle fibers, as in the absence of TTX, was much higher at 27°C than at 37°C. Such an increase in membrane conductance on cooling was not observed in muscle fibers from normal subjects.

Figure 3E shows that in TTX-containing, Cl-free solution at 27°C the membrane characteristics of paramyotonic fibers were similar to the characteristic of the normal fiber and similar to all characteristics measured in the same solution at 37°C. In this solution, the membrane current is mainly carried by potassium ions. Potassium current therefore seems unaffected by temperature both in normal subjects and in paramyotonic patients. The cold-induced increase of membrane conductance of paramyotonic fibers in the presence of TTX must therefore be caused by an increased g_{Cl}, and the cold-induced depolarization in the absence of TTX by an increased g_{Na}.

In summary, at 37°C all component conductances of paramyotonic fibers were found to be normal except for a reduced g_{Cl} in the patient who also had myotonia in a warm environment. When paramyotonic fibers were cooled, g_{Na} and g_{Cl} were increased while g_K was not altered.

Fiber Diameters and Specific Capacitances. To test our analysis of the electrophysiological data, we made a statistical comparison of histologically evaluated fiber diameters, d_h, and the diameters calculated from the voltage clamp data, d_c. There was no significant difference between d_h and d_c. The mean d_h and d_c values ± SD *(n)* in μm were 71.1±9.55 (40) and 63.4±6.7 (12) for patient PWM D, and 66.9±8.26 (40) and 68.3±10.5 (24) for patient PWOM B, respectively. Paramyotonic d_h did not significantly differ from normal d_h, which was 68.9±11.7 (120). At 27°C, d_c was 68.8±13.2 (5) for patient PWOM A, and 57.8±10.0 (5) for patient PWM D. For patient PWOM B, d_c was 91.1±20.0 (7) at 21°C.

Paramyotonic membrane capacitances were 4.8 ±1.6 (19) μF/cm² (PWM D) and 3.6±1.3 (11) μF/cm² (PWOM B). The normal value was 3.6± 1.3 (11) μF/cm², indicating that this parameter is not affected in paramyotonia.

Recovery from the Paralyzed State. Preparations which had been cooled to 27°C in TTX-free solution and had become paralyzed, i.e., were depolarized and had high membrane conductances, did not recover for up to 2 hours when rewarmed to 37°C in a TTX-containing solution.

An exceptional observation was made with a preparation from patient PWOM B which had been stored overnight at 8°C in TTX-containing solution. After rewarming to 21°C, this preparation showed high resting potentials and normal membrane conductances (123±24 μS/cm², n = 9). This is remarkable because the membrane conductance must have been increased for at least some time after the original cooling. As this preparation was put in TTX-free solution at 21°C to test the excitability, the fibers began to twitch and continued doing so for 5 to 10 minutes. After this period of time, all of the fibers had depolarized to about −40 mV.

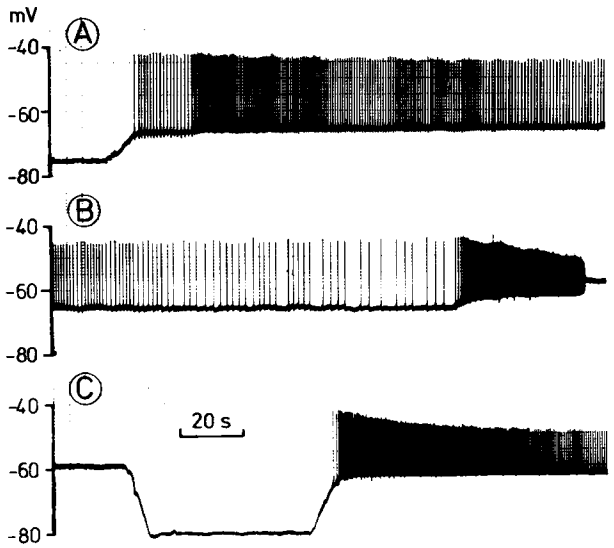

Figure 4. Electrical activity in a partially voltage-clamped fiber from a patient with paramyotonia without myotonia in a warm environment (PWOM B) in Cl-free solution at 21°C.

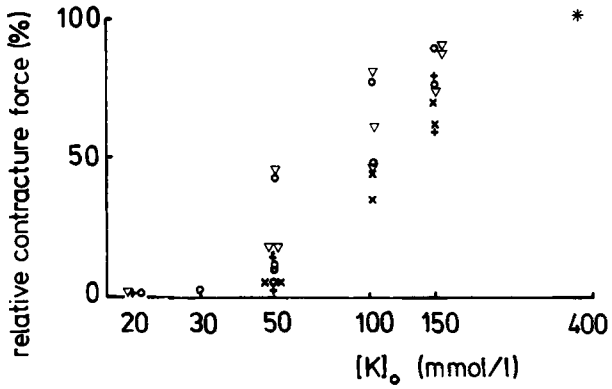

Figure 5. Dependence of peak of contracture force on the external potassium concentration in 2 patients with paramyotonia (PWOM B [x] and PWM D [+]) and 2 normal subjects. For each set of results, the contracture amplitude at 400 K was set at 100%.

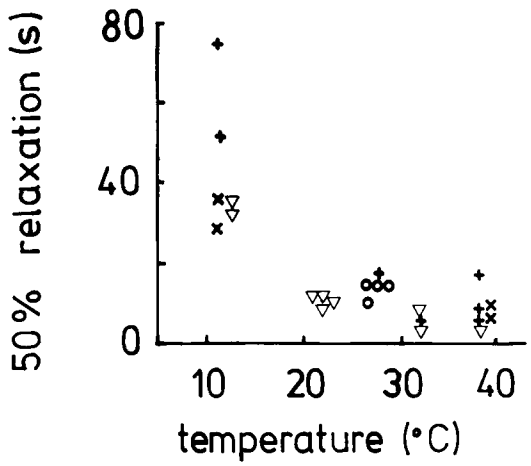

Figure 6. Time needed for 50% relaxation of contractures elicited by K, 150 mmol/liter, in muscles from 2 patients with paramyotonia (PWOM B [x] and PWM D [+]) and 2 normal subjects, at various temperatures.

With such a "paralyzed" preparation, we could show that excitability and the capability of twitching can be restored by artificial repolarization. The experiment is illustrated in Figure 4 and was carried out in the following way. A fiber was impaled with 3 electrodes, and the clamp amplifier was turned on with a gain just sufficient to clamp the membrane potential to various levels, but not sufficient to suppress the generation of action potentials. When the membrane potential was clamped to −80 mV for some time and was then slowly lowered, the fiber started to produce action potentials at −63 mV. The amplitude of these action potentials was reduced, presumably by the clamp. The frequency of the action potentials could be increased by further lowering of the clamp potential. When the clamp potential was set to −40 mV, spontaneous activity ceased and the fiber was "paralyzed." Renewed repolarization to −80 mV and depolarization to threshold showed that the fiber was able to generate action potentials again.

Paralysis thus can be removed by membrane repolarization. We have no indication, however, of how normal g_{Cl} and g_{Na} are restored in patients who recover from the paralyzed state.

Potassium Contracture Experiments. There appeared to be no difference between the results obtained with paramyotonic and normal muscles. The threshold concentration of K at which contractures appeared was 40–50 mmol/liter, and maximum force was attained at a K concentration of 400 mmol/liter (Fig. 5). Since a complete depolarization was presumably caused already at a K concentration of 150 mmol/liter, this result suggests that factors like diffusion of K, synchronization of the response, and the rate of inactivation also determined the peak of K contractures.[14] The time course of the force development during the application of 150 mmol of K/liter was such that relaxation was 50% complete within 5 to 20 seconds in both paramyotonic and normal muscles. The time course of the contractures was unaltered by reducing the temperature from 38°C to about 25°C (Fig. 6). Recovery of the contracture ability in control solution (4 mmol of K/liter) was good in all muscles (14%–24% of control peak force after 1

minute). Thresholds, force–K concentration relationships, and recoveries similar to those found here were also found in fast-twitch white muscles of rats.[5,14]

It was of special interest to see whether the paramyotonic muscle stiffness could be due to tonic contractures. The latter would be expected to occur in muscles in which the threshold potential for a contracture is lower than that for recovery. None of the muscles studied was able to recover in a solution containing 20 mmol of K/liter. Considering the high contracture threshold (>40 mmol/liter), it is therefore not surprising that no tonic contractures were produced by applying 20–50 mmol of K/liter for up to 3 minutes.

The recovery of the contracture ability, measured by the contracture force following a 1-minute wash in a low K solution, was on the average 24.2% (range 3%–60%) in all muscles tested. Effects of temperature (range 22°C–38°C) were small, and no difference between normal and paramyotonic muscles was found. The recovery ability was not altered by reducing the K concentration of the washing solution from 4 to 1 mmol/liter and was only slightly impaired by increasing the K concentration to 8 mmol/liter.

An observation which was consistently made was that all the effects of cooling down to 10°C for up to 5 minutes were fully and rapidly reversible at room or at body temperature. From these results we conclude that the electromechanical coupling and contractile apparatus in paramyotonic intercostal muscle is not different from that in normal muscle.

Pharmacological Experiments. Acetylcholine (20 mg/liter; 110 μmol/liter) never caused force development in any of the muscles investigated. This suggests that the motor innervation of paramyotonic muscles is not impaired.

All paramyotonic muscles tested by 20 or 50 mmol of caffeine/liter answered by a prompt force development probably reaching maximum force. Measurable force was produced by less than 5 mmol of caffeine/liter. Similar results were obtained with normal muscles, indicating that the ability of caffeine to release Ca from the sarcoplasmic reticulum is not reduced in paramyotonic muscles.

DISCUSSION

The major result of our studies is the identification of the muscle cell membrane as the site of pathological alteration in paramyotonia congenita, and the elucidation of the temperature dependences of the component conductances, which are rather abnormal in the case of g_{Na} and of g_{Cl} (see Table 1).

At 37°C, we found normal resting potentials and normal membrane characteristics in fibers from patients with paramyotonia who had no myotonia in a warm environment. This is in agreement with the clinical experience that these patients are asymptomatic in a warm environment. Fibers from the patient who had myotonia in a warm environment showed normal resting potentials, but the membrane conductance was reduced. The altered conductance was found to be caused by a reduced g_{Cl}, and this again fits with the clinical finding that this patient shows myotonic symptoms in a warm environment. Reduced g_{Cl} is well known as the pathogenetic basis of myotonia, at least in myotonia congenita[12] and in experimentally induced myotonia.[17]

On cooling, all paramyotonic fibers slowly depolarized from −80 mV to about −40 mV. This event is most likely the cause of the clinical symptoms, such as fibrillation, muscle weakness, and paralysis. At the beginning of cooling, when the depolarization reached the electrical threshold, repetitive activity started which differed from myotonic after-activity in that the paramyotonic runs had lower frequencies (~2 Hz) and lasted several minutes instead of seconds. These runs probably correspond to the light muscle twitching reported by some patients. With continued cooling, depolarization reached a level at which repetitive firing stopped because the fibers became inexcitable. Clinically, this corresponds to the phase in which the muscles become paralyzed. The slowed muscle relaxation and the resistance against passive stretch described in the foregoing paper[7] are not sufficiently explained by our in vitro results. They could be caused by spontaneous activity in muscle fibers of agonists and antagonists, but the electromyographic evidence does not support this view. Our small preparations, paramyotonic and normal, never showed long-lasting contractures, either in physiological solution or with elevated levels of external potassium. Prolonged contractures were produced only in the presence of caffeine, but the properties of these contractures were not different in normal and paramyotonic muscles. Thus, paramyotonic muscle stiffness needs further investigation.

The resting potential of a muscle fiber is determined by the ratio of g_K to g_{Na}. The Cl conductance is irrelevant since Cl is passively distributed between inside and outside the cell membrane.[15] The cold-induced depolarization could therefore

be caused by an increase in g_{Na} and/or by a decrease in g_K. Prevention of depolarization by the Na channel blocker TTX indicated that depolarization was caused by an increase of g_{Na}. In TTX-containing, Cl-free solution, the membrane current is mainly carried by K ions. In this solution the membrane characteristics were almost identical at 37°C and 27°C and were not different from the normal ones. This result indicates that the temperature change had no effect on g_K.

The mechanism of the increase in g_{Na} is unknown. It is possible that the Na channel does not close properly after it has been opened in the cold. Muscular activity accelerates the progress of depolarization presumably by allowing Na channel opening. If this reasoning is correct, a drug blocking open Na channels should be effective in the therapy of paramyotonic symptoms. Indeed, some of us had excellent success in preventing paramyotonic symptoms in our patients by using the lidocaine derivative tocainide, a Na channel blocker.[16]

At low temperature the membrane conductance of paramyotonic fibers is much higher than in normal fibers, and this was found to be due to their increased g_{Cl}. An increased g_{Cl} at 27°C was also found in fibers from patient PWM D in which g_{Cl} was reduced at 37°C. An increased g_{Cl} on cooling (Q_{10} = 0.43) has also been reported for myotonic goat muscle.[11]

The paramyotonic changes of g_{Cl} and g_{Na} could be coupled. The finding of an increased g_{Cl} at low temperature when depolarization did not occur in the presence of TTX does not exclude the possibility that in this condition the blocked Na channel was also altered. During recovery, g_{Na} and g_{Cl} seem to return to their original values with differing time courses. In patient PWM D, we observed clinically that after exposure to cold and rewarming, the mechanical and electromyographical signs of myotonia are missing for several hours. This suggests that after rewarming it takes a long time for g_{Cl} to return to a low value whereas g_{Na} is already normal. The slow recovery of both g_{Na} and g_{Cl}, which is reflected in the protracted muscular weakness after an extended exposure to cold, suggests that the alterations in both types of ionic channel are severe and perhaps can only be reversed by the production of new pores.

The experiments in which excitation-contraction coupling and the contractile apparatus were tested did not reveal any pathological alterations in fibers from either patient PWOM B or patient PWM D and this was so both at 37°C and at low temperature. In particular, muscle relaxation seemed normal and there was no membrane potential value at which the muscles were able to produce long-lasting contractures. It is possible, however, that the clinical findings of slowed relaxation and resistance against stretch do not apply to the external intercostal muscle.

Fortunately, other workers have also used the external intercostal muscle, so our control values can be compared with their findings. In this study, the resting potential values were more negative and the specific membrane conductances were lower than the corresponding values of previous investigations.[6,12,13] These differences may be explained by the lower K concentration (3.5 mmol/liter) in our bathing solution. With 4.5 mmol of K/liter, we obtained resting potentials and membrane conductances which were in the same range as those found by the above-mentioned authors. The good agreement of our calculated fiber diameters with the histological data supports our belief that the method of determining membrane parameters[2] was correct and that the assumption of R_i = 125 $\Omega \cdot cm$[13] for the unknown specific myoplasmic resistivity of paramyotonic muscle was valid.

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