CHARACTERISTICS OF Na⁺ CHANNELS AND Cl⁻ CONDUCTANCE IN RESEALED MUSCLE FIBRE SEGMENTS FROM PATIENTS WITH MYOTONIC DYSTROPHY

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SUMMARY

1. Electrical and contractile properties of resealed fibre segments were investigated by a variety of *in vitro* techniques. The preparations were removed from skeletal muscles of normal subjects and of eight patients with myotonic dystrophy.

2. Several hours after removal, fibre segments from normal subjects and those patients in whom myotonia was the primary symptom had resting membrane potentials of approximately -80 mV. In contrast, fibre segments obtained from patients in whom muscle dystrophy was more expressed were depolarized (-60 to -70 mV).

3. Contractions induced in fibre segments of myotonic muscle which had normal potentials were characterized by slowed relaxation which was due to electrical after-activity.

4. After single stimuli, long-lasting (3–100) runs of action potentials were recorded intracellularly from the myotonic muscle. In some of these fibre segments complex repetitive discharges were observed; multiple sites of locally gated currents were identified.

5. The three-electrode voltage clamp was used to determine the total membrane conductance, g_m, and the ion component conductances. All fibres of a particular patient had similar conductances. However, the Cl⁻ conductance varied from patient to patient from normal (74% of g_m) to low values (30% of g_m). The K⁺ conductance was normal in all fibres of all patients.

6. The patch-clamp technique was used to record currents through single Na⁺ channels of the sarcolemma. After treatment of the fibre segments with collagenase gigaohm seals were routinely obtained. The rate of success was greater when using the cell-attached mode than the inside-out mode.

7. Sodium channel currents were elicited by depolarizing voltage steps which produced an initial burst of Na⁺ channel openings. Up to ten channels were activated simultaneously when the patch was depolarized to potentials more positive than -30 mV. The Na⁺ channels re-opened very rarely in controls. The macroscopic sodium current, I_{Na}, was reconstructed by averaging depolarizing pulses. The time

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constant of rapid decay of I_{Na} reflecting macroscopic inactivation, the onset of I_{Na} and the amplitude of I_{Na} were voltage dependent. The mean amplitude of the current produced by re-openings was on average only $0.11 \pm 0.04\%$ of the amplitude of the peak current.

8. Late openings of the Na^+ channels were frequent in patches on the myotonic fibre segments. The amplitude of the current produced by re-openings was as high as about $0.75 \pm 0.11\%$ of the amplitude of the peak current. These re-openings were apparently unrelated to the reduced Cl^- conductance because they were observed in fibre segments of patients with normal Cl^- conductance and were not detected in control muscle which was treated with the Cl^- channel blocker 9-anthracene carboxylic acid. The open time of the re-openings varied between 0.7 and 0.8 ms at all potentials.

9. We conclude that a reduced Cl^- conductance is not essential for the production of myotonia in myotonic dystrophy. Thus, the hyperexcitability in those cases where Cl^- conductance was normal may be solely due to the re-openings of Na^+ channels.

INTRODUCTION

Although myotonic dystrophy is the most common hereditary muscle disease in adults, there are relatively few studies of the electrophysiological properties of the sarcolemma (for a review see Rüdel & Lehmann-Horn, 1985). Moreover, the available data are somewhat conflicting. For example, Hoffmann & Rowe (1966) and Gruener, Stern, Markovitz & Gerdes (1979) found the resting membrane potential to be 10–15 mV lower than normal, whereas Lipicky (1977) reported a high resting potential and a reduced intracellular Na^+ concentration. There are few data on the chloride conductance in myotonic dystrophy. In two patients this conductance was reduced to 50% of the total membrane conductance, but in four others it was unchanged (Lipicky, 1977).

An altered sodium current has been shown in muscle preparations from patients with either hyperkaliaemic periodic paralysis (Lehmann-Horn, Küther, Ricker, Grafe, Ballanyi & Rüdel, 1987) or paramyotonia congenita (Lehmann-Horn, Rüdel & Ricker, 1987), in myoball cultures from patients with various myotonic disorders (Rüdel, Ruppersberg & Spittelmeister, 1989) and in muscle preparations from myotonic goats (Bryant & DeCoursey, 1980). However, there are limited studies concerning fast Na^+ current in diseased muscle. The reason may be that the fast Na^+ current is difficult to resolve with the conventional voltage-clamp techniques and that intact muscle fibres were previously thought to be necessary for such investigations. Furthermore, although the approach to measure fast sodium currents on skeletal muscle with the loose patch-clamp technique has been somewhat successful (Almers, Roberts & Ruff, 1984), it has not yet been used for diseased muscle.

It has been reported previously that fibre segments of skeletal muscle biopsies subsequently reseal, possess normal membrane properties (Lehmann-Horn & Iaizzo, 1989) and are suitable for the patch-clamp technique (Franke & Hatt, 1989). Therefore, it is possible to use such specimens of routinely performed muscle biopsies obtained under local anaesthesia for a number of electrophysiological investigations. It seemed therefore of great interest to study the electrophysiological properties of

skeletal muscle from patients with myotonic dystrophy. Alterations of Na^+ channels were found in a patient with chondrodystrophia myotonica (Lehmann-Horn, Iaizzo, Franke, Hatt & Spaans, 1989).

<div align="center">METHODS</div>

Muscle specimens were dissected under local anaesthesia from patients with myotonic dystrophy. Patients who had to undergo an *in vitro* test for susceptibility to malignant hyperthermia, but had a negative test result, served as controls. Electromyograms (EMGs) were recorded from the biceps bracchii and the vastus medialis muscles. The muscle with the more pronounced signs of electrical myotonia was chosen for biopsy. Additionally, *in vitro* EMGs and force were simultaneously recorded as previously described (Ricker, Rüdel, Lehmann-Horn & Küther, 1986). Although they were variable, clinical and electrical signs of myotonia were found in all patients ($n = 8$; see Table 1).

The skin incision was about 3 cm long, and the removed specimen measured about 4 cm. In all cases, attempts were made to obtain a specimen that included the endplate region. Muscle bundles with diameters of 2–3 mm were prepared from the specimen which were used for the various experiments. All procedures were in accordance with the Helsinki convention and were approved by the Ethics Commission of the Technical University of Munich.

Solutions

The standard solution used for transportion, dissection and electrophysiological experiments contained (in mM): NaCl, 108; KCl, 3·5; $CaCl_2$, 1·5; $MgSO_4$, 0·7; $NaHCO_3$, 26·2; NaH_2PO_4, 1·7; sodium gluconate, 9·6; glucose, 5·5; sucrose, 7·6 (315 mosM). The Cl^--free solution, used in some of the voltage-clamp experiments, was made by replacing NaCl and KCl with the respective methane sulphonate salts, by replacing $CaCl_2$ with calcium gluconate and by omitting the sugars in order to avoid hyperosmolarity. All solutions were maintained at 37 °C if not indicated otherwise. The pH was adjusted to 7·4 by gassing the solutions with 95 % O_2 and 5 % CO_2. Some solutions contained tetrodotoxin (TTX; 1 μM).

Electrical membrane properties

Resting and action potentials were recorded from the resealed fibre segments by means of capacity-compensated microelectrodes. Voltage-clamp experiments were performed on the same fibres with three microelectrodes in either normal or Cl^--free solutions (Lehmann-Horn, Rüdel, Dengler, Lorkovic, Haass & Ricker, 1981). Data were collected and analysed with a computer system (Digital Equipment Corporation, Maynard, MA, USA).

Patch-clamp experiments

Bundles of fibre segments were superfused for up to 2 h with standard solution containing 1–2 mg/ml collagenase (Sigma, Type Ia). After this treatment the standard patch-clamp technique was employed (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Patch-clamp pipettes were pulled with a microprocessor-controlled device (DMZ puller, Zeitz, Augsburg, FRG). After fire-polishing they had a diameter of about 1 μm. Inside-out patches were moved at the tip of the patch-clamp pipette to a recording chamber which could be perfused separately (Franke, Hatt & Dudel, 1986; Franke & Hatt, 1989). This was necessary, because the collagenase-treated muscle was very sensitive to changes in the ionic composition of the bathing solution. The 'intracellular' solution with which the sarcoplasmic side of an inside-out patch was perfused contained (in mM): KCl, 150: $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 10, HEPES, 10; pH 7.2. In one set of experiments, the Cl^- channel blocking agent 9-anthracene carboxylic acid was added to the solution in the patch-clamp pipette in an amount sufficient to provide a final concentration of 0·05 mM (Bryant & Morales-Aguilera, 1971; Furman & Barchi, 1978; Kwiecinski, Lehmann-Horn & Rüdel, 1988).

The data were stored on a modified PCM recorder (44 kHz digitization rate) and evaluated off-line with the programs described previously (Franke *et al.* 1986; Franke & Dudel, 1987). These programs constructed 'idealized' records by averaging subsequent data points whose difference was smaller than a fixed threshold. The 'idealized' records were also stored for further evaluation. The capacitive artifacts were compensated either digitally by subtracting averages of traces without channel activity or by an analog compensation when there was a large number of channels

under the patch. This latter method of compensation proved to be very effective because it could be adjusted immediately to the potentially changing conditions of an experiment.

<div align="center">RESULTS</div>

Membrane potentials

Several hours after removal, fibre segments obtained from the normal subjects and those patients in whom myotonia was the primary symptom had normal resting

TABLE. 1. Membrane potentials, membrane conductance and current produced by late openings of Na^+ channels of patients and of controls

Patient code	Electrical myotonia		Resting membrane potential (mV)$\pm$s.d.	R_m (Ω cm^2)	g_{Cl} (%)	Proportion of late current (%)$\pm$s.d.
	In vivo	In vitro				
MyD 10	+	+/++	-72.6 ± 9.2 (16)	3820 (6)	70	*
MyD 11	+	−	-66.4 ± 4.4 (8)	5520 (7)	74	0.53 ± 0.11 (3)
MyD 12	+	−	-60.0 ± 2.9 (8)	10470 (4)	58	*
MyD 13	+++	++/+++	-82.7 ± 6.6 (20)	3660 (8)	70	0.71 ± 0.08 (5)
MyD 14	++	++	-79.9 ± 8.7 (23)	14210 (8)	31	0.9 ± 0.13 (3)
MyD 15	++	++	-77.4 ± 4.4 (11)	13980 (10)	17	0.85 ± 0.17 (3)
MyD 16	++	++	-80.6 ± 3.4 (41)	11030 (11)	44	*
MyD 17	+	++	-73.4 ± 4.0 (10)	10900 (7)	44	0.78 ± 0.1 (4)
Controls	−	−	-80.4 ± 2.1 (82)	3800 (10)	76	0.11 ± 0.04 (12)

Number of fibres or number of patches are given in parentheses.

− = none, + = little, ++ = moderate and +++ = strong electrical myotonia recorded either in vivo or in vitro; / = intermediate values. * = no data.

membrane potentials (MyD 13–16; see Table 1). In contrast, the fibre segments obtained from patients in whom muscle dystrophy was more expressed were depolarized (MyD 10–12, 17; see Table 1).

Myotonic activity either occurred spontaneously or was induced by the impaling microelectrode. This electrical activity was totally blocked by TTX (1 μM). At the beginning of a myotonic burst, propagated action potentials occurred repetitively. The amplitude of these potentials was the same along the fibre segment. Subsequently, the fibre membrane slowly depolarized. The width of the action potentials increased until the potential eventually became multiphasic (Fig. 1). Up to three different peaks were recorded (e.g. see Fig. 1). This behaviour may be best explained by different local potentials which were activated from distant sites in the muscle fibre membrane. Figure 1 shows these spontaneous changes in the membrane potential recorded from the same fibre by two intracellular microelectrodes at different locations (V_1 and V_2). In this case three different generators were detected (1, 2 and 3). They each had a fairly constant firing rate which was similar but not identical. At one point in time (II), potentials 2 and 3 fired synchronously. At this moment, the amplitude of the resulting potential was larger than at times when the potentials (2 and 3) were separated (e.g. time points I and III). In addition, the amplitude of the three potentials depended on the recording site. For example, in the

lower panel of Fig. 1 potential 3 was higher than potential 2 at location V_1 but was smaller than potential 2 at location V_2. This pattern was not dependent on the order in which the potentials occurred (e.g. at time points I and III). The amplitude of the respective potentials depended on the distance of the recording electrode to the local generator.
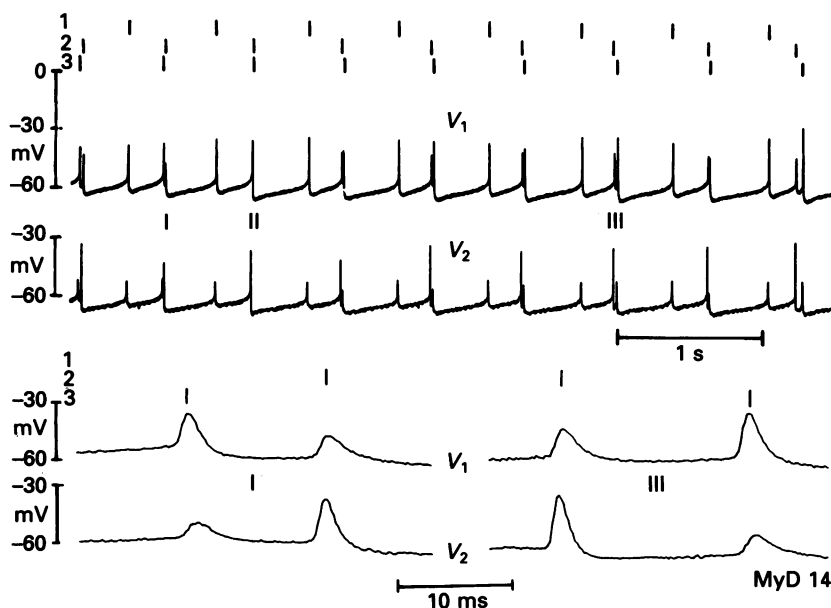

Fig. 1. Intracellular recordings of spontaneously firing action potentials of resealed fibre segments of a patient with myotonic dystrophy (MyD 14). Simultaneous recordings of two intracellular microelectrodes at different locations are shown (V_1 and V_2). Action potentials split up: such a situation is shown below in better time resolution at times I, II and III. The action potentials were obviously elicited at three different positions and fired out of phase, as indicated above.

Current–voltage relationship

In a normal solution in the slope of the steady-state current–voltage relationship reflects the membrane conductance, g_m. In addition, to calculate the ionic component conductances, the current–voltage relationships were determined in Cl^--free solution which contained TTX. The slope of the current–voltage relationship determined in this solution reflects that K^+ conductance, g_K, which was normal in all myotonic fibres. The difference between the two curves (normal and Cl^--free solutions) represents the voltage-dependent chloride current density which is proportional to the chloride conductance, g_{Cl}. In half of the patients (MyD 14–17) the current–voltage relationship had a low slope reflecting a decreased membrane conductance, g_m (Fig. 2). The resting Cl^- conductance was reduced to values between 30 and 44% of g_m (Table 1). In the other patients both the membrane and Cl^- conductances were

normal (MyD 10, 11, 13) or only slightly reduced (MyD 12; see Table 1). In the EMG recorded *in vivo* and *in vitro* from one of these latter patients (MyD 3) electrical signs of myotonia were most pronounced.

Patch-clamp experiments

After the treatment with collagenase, it was possible to obtain gigaohm seal recordings in five out of seven preparations. However, the rate of success differed
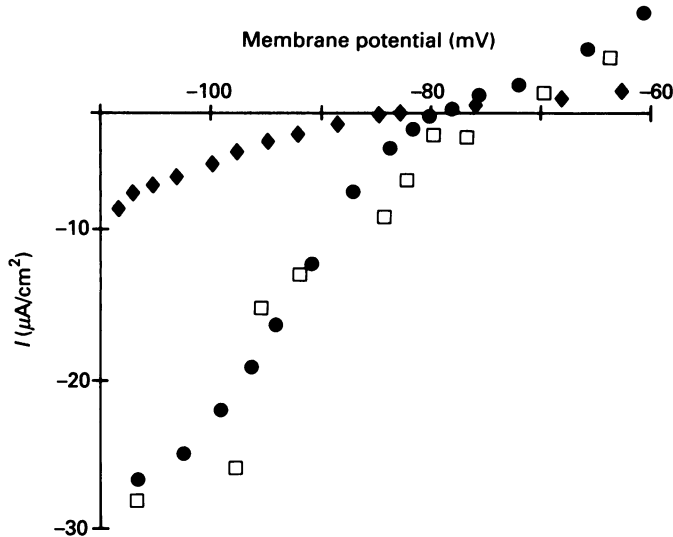

Fig. 2. Current–voltage relationships of resealed fibre segments from two different patients with myotonic dystrophy and a control. The data were obtained in normal solution. The curve for one patient (□, MyD 13) was similar to the curve for a normal control (●), whereas for the other (◆, MyD 14) the decreased slope represented a lower Cl⁻ conductance.

greatly from one muscle preparation to the next. In some preparations ten to twenty pipettes were used in order to obtain one gigaohm seal, whereas in other preparations it was possible to obtain a seal with almost every attempt. As the number of patients available for the study was limited, it was imperative to collect as many data as possible from every patient. Thus, if we were not able to record channel activity from a first bundle, we treated a second or third one with collagenase. Adequate recordings from at least three gigaohm seals, in the cell-attached mode, were obtained from five of the patients. The data collected from the other three were not included, because the number of recordings was too small.

After appropriate recordings were obtained in the cell-attached mode, the membrane potential of the fibre was determined. The effective potential was calculated as the sum of the membrane potential and the patch potential. Our rate of success was lower when we used the inside-out mode, because of a break-down of the gigaohm seal during excision of the patch. We evaluated only patches without K^+ channel activity.

Na^+ channels of normal muscle fibre segments were previously investigated in the

inside-out mode (Franke & Hatt, 1989). Since we investigated the diseased muscle primarily in the cell-attached mode, we present first control data obtained in the cell-attached mode in the following.

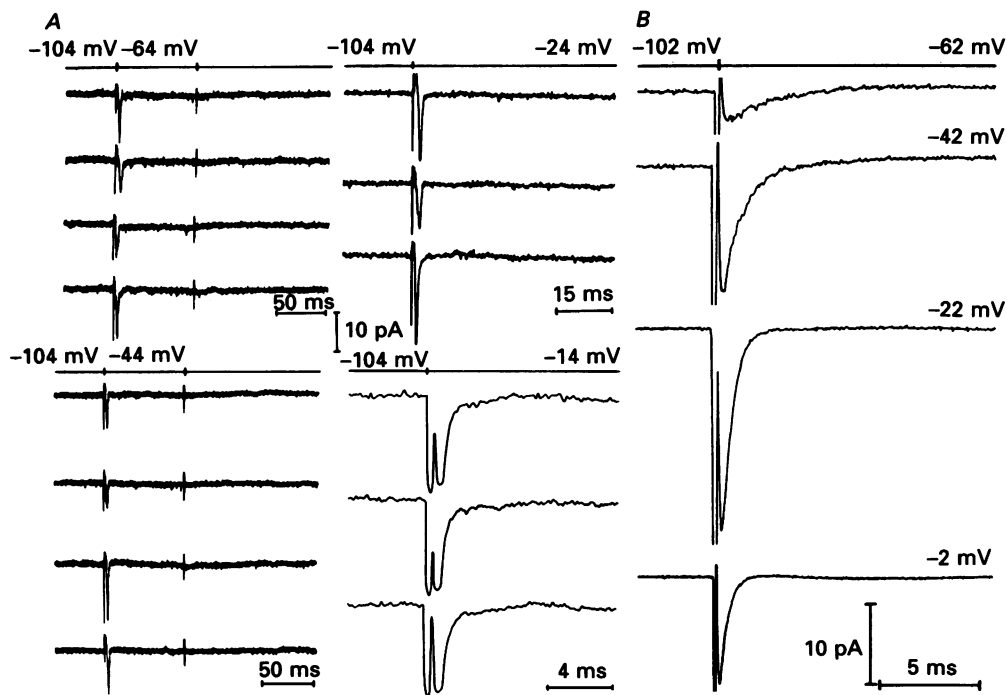Figure 3*A* shows data recorded from a cell-attached patch of control muscle.

Fig. 3. Recordings in the cell-attached mode from normal controls (vastus medialis muscle). *A*, recordings of Na$^+$ channels elicited by depolarizing pulses of different amplitudes. Holding potential -104 mV. Duration of depolarization pulse was 80 ms. Note the different time scales. Low-pass filter at 3 kHz. *B*, Na$^+$ currents reconstructed by averaging 50–250 single-channel recordings at each potential in the cell-attached mode. The patch was depolarized from a holding potential of -102 mV by voltage steps of different amplitude. The recordings were low-pass filtered at 10 kHz.

Sodium channel openings were elicited by depolarizing the membrane in 20 mV steps from a holding potential of -104 mV. Up to ten channels were present in one patch and these channels were activated within a few milliseconds. If numerous such records were averaged, a reconstructed macroscopic I_{Na} was obtained which was similar to that recorded by conventional voltage-clamp technique (Campbell & Hille, 1976). For example, Fig. 3*B* shows macroscopic currents derived from 50–250 depolarizing pulses recorded from the same patch.

The Na$^+$ channels of normal muscle were not affected by the presence of the Cl$^-$ channel blocker 9-anthracene carboxylic acid in the patch electrode. In its presence, recordings identical to those shown in Fig. 3 were obtained.

Following a depolarization step from the same holding potential, the average number of channels activated in cell-attached patches was larger than the number activated in inside-out patches (Franke & Hatt, 1989). This may indicate that the

size of the cell-attached patches was larger. Although not systematically investigated, as reported by others (Almers *et al.* 1984), we noted that the number of channels located within a patch was dependent on the location of the patch along the fibre. For example, the number of channels in a patch was larger if the fibre segment contained the endplate and the patch-clamp pipette was positioned near to it.
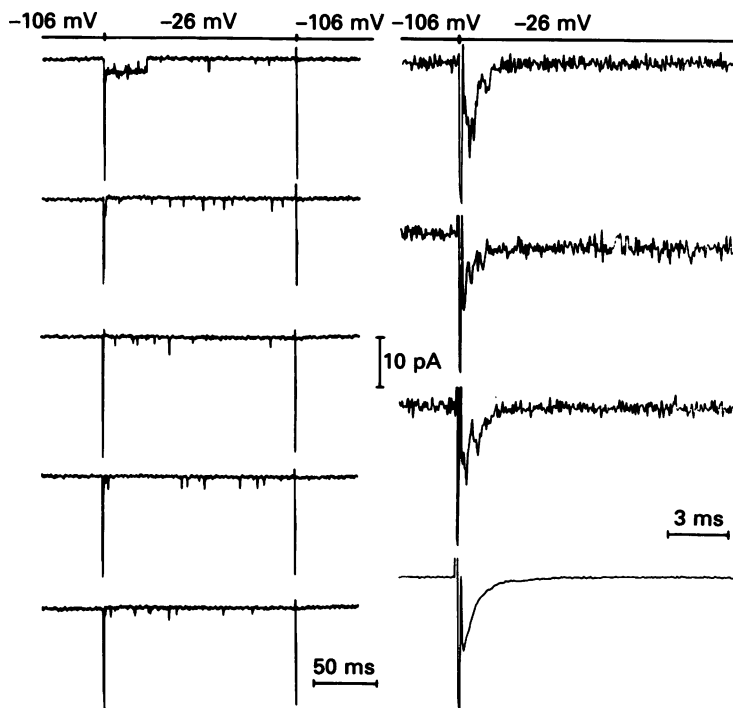

Fig. 4. Recordings obtained from a patch of muscle fibre segment taken from a patient with myotonic dystrophy (MyD 12). In the left part of the figure, five consecutive records are shown. Late openings of channels can be observed regularly throughout the depolarizing pulse. Low-pass filter at 2 kHz. On the right, records of the same patch are low-pass filtered at 10 kHz and are shown on an extended time scale. The average of sixty-eight such records is shown below.

Records of Na^+ channel activity from patches with gigaohm seals formed on diseased fibres are shown in Fig. 4. Subsequent to the early current, which was elicited within 1–3 ms after the beginning of the depolarization step, channels regularly opened late in these patches throughout the depolarization step. Figure 4 shows five consecutively obtained records. The early current can be best discriminated in unfiltered records with a higher time resolution shown on the right.

Macroscopic currents were reconstructed in the same way as for the control data. The time constant of the rapid decay for the reconstructed macroscopic currents for patches of diseased muscle did not differ significantly from controls, as shown in Fig. 5.

We evaluated the late openings that occurred in each depolarization step, produced 'idealized records' (Franke *et al.* 1986) and averaged them. We were then able to calculate their contribution to the macroscopic current. As an example,

Fig. 6 shows the evaluation of the data obtained from a patch on a diseased fibre: the 'idealized records' (above) and the corresponding average (below) of 100 'idealized records'. The average amplitude of the current produced by the late openings at about -25 mV was then related to the amplitude of the peak of the macroscopic
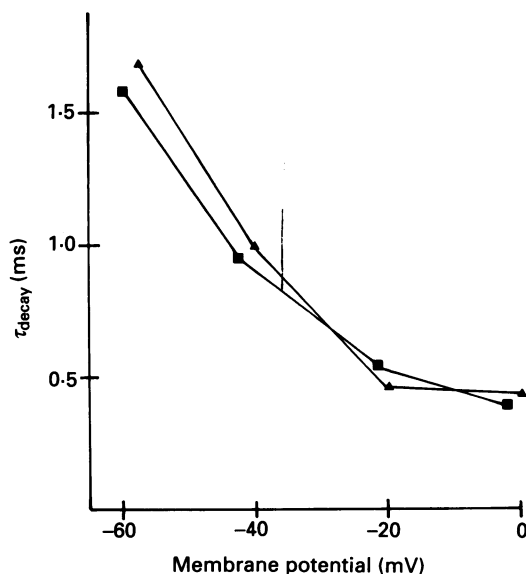

Fig. 5. Evaluation of time constants of decay of the reconstructed macroscopic Na^+ current I_{Na}. I_{Na} was reconstructed by averaging at least fifty records at the different potentials. Data were obtained from a normal control (■) and from a patient (▲, MyD 14).

current. A comparison of these values is provided in Table 1 for the patches of control and of diseased muscle. The average amplitude of the current produced by late openings in diseased muscle was 0·75 % of the amplitude of the peak current at potentials of about -25 mV. The value for controls was 0·11 %, a value which was lower than that previously reported for inside-out patches of control muscle, 0·5 % (Franke & Hatt, 1989). Therefore, it seems that the excision of the membrane patch had an effect on the rate of late Na^+ channel openings. Unfortunately, we were not able to record from the same membrane patch in the cell-attached and then in the inside-out mode. It was nearly impossible to excise a patch after it was for some minutes held in the cell-attached mode. Nilius (1988) studied the gating behaviour of cardiac sodium channels in the cell-attached mode and after excision. He found a distinct delay in the decay of averaged currents in excised patches due to the appearance of long-lasting openings or bursts of openings.

Openings were frequently observed in diseased muscle when the patch was repolarized to values between -60 and -80 mV. In the subsequent depolarization, the number of channels available for activation was small due to the inactivation of Na^+ channels at rest. Therefore, we used holding potentials more negative than -100 mV. After a patch was repolarized to holding potentials more negative than -100 mV, openings of Na^+ channels were not observed.

The average open time of late openings and its dependence upon the membrane

potential was evaluated in Fig. 7. The mean open time of the Na^+ channels on the myotonic fibres was independent of the potential in contrast to inside-out patches of control muscle.

In one patient (MyD 14) we were able to measure both in the on-cell and the inside-
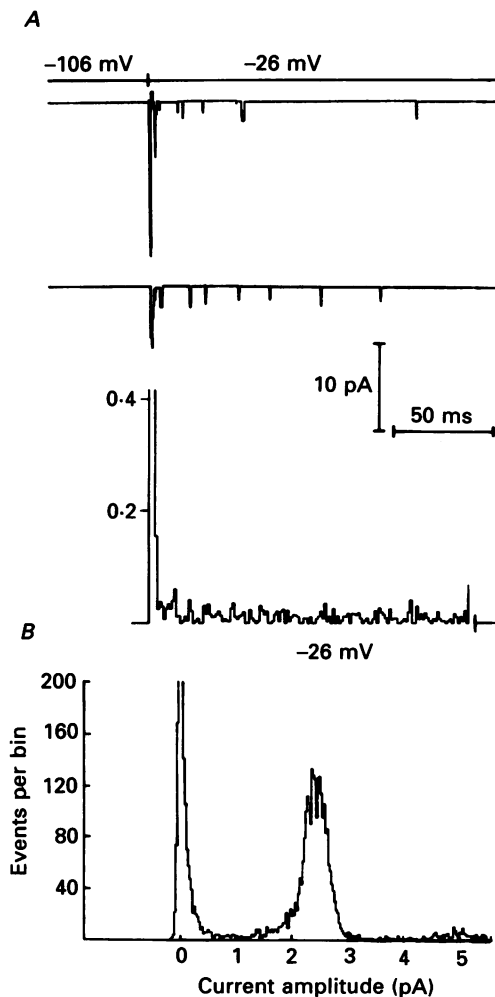

Fig. 6. Evaluation of late Na^+ channel openings. The channel openings were elicited by a depolarizing pluse of amplitude 80 mV. Recordings are from a patient (MyD 14). *A*, two examples of 'idealized records' evaluated by the computer program are shown above and below the average of 100 such records. The ordinate shows the number of open channels in the respective time bin. Binwidth 2 ms. *B*, evaluation of single-channel current amplitudes of late openings. Binwidth 0·05 pA.

out modes from different patches of the same preparation. After formation of an on-cell patch, the pipette was immediately lifted off the cell and the membrane vesicle at the tip of the electrode was moved to the separate recording chamber. When the vesicle was exposed to air once or several times (Hamill *et al.* 1981) an inside-out patch was eventually established. Figure 8 shows a recording in the inside-out mode, in which the capacitive artifact occurring after the beginning of the depolarization

was compensated. The rate of late openings was increased also in this recording mode (3·8% compared to 0·5% in controls; see Franke & Hatt, 1989). The time constant of rapid decay of the macroscopic current reconstructed from data obtained in the inside-out mode was also unchanged compared to controls (not shown).
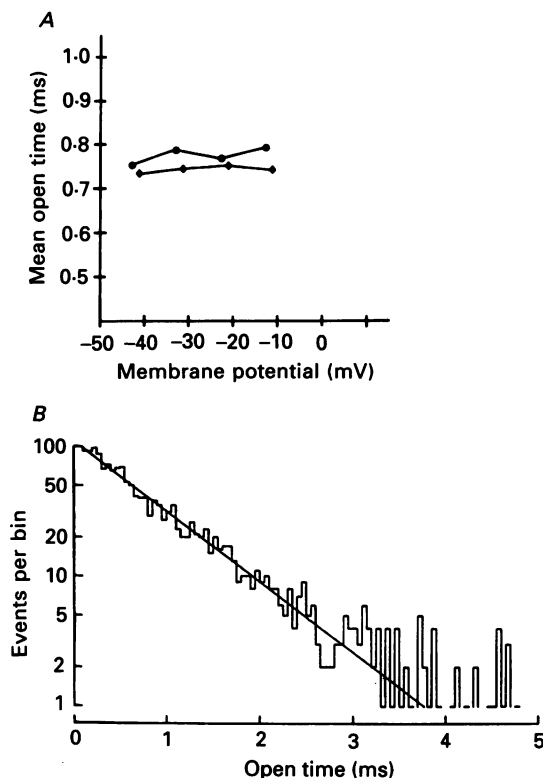

Fig. 7. Evaluation of open times of late channel openings. A, the mean open time was determined at different membrane potentials; each point represents the evaluation of at least 1500 openings. Data from two different patients are plotted. B, histogram of durations of late single-channel openings elicited by depolarizing pulses to -22 mV. Ordinate is a logarithmic scale. The distribution is fitted with a single exponential with a time constant τ of 0·74 ms; binwidth 50 μs.

DISCUSSION

We point out here that sarcolemmal abnormalities in the inactivation of Na^+ channels alone or in conjunction with a reduced Cl^- conductance are responsible for the myotonic activity which we recorded either *in vivo*, extracellularly *in vitro* or intracellular *in vitro*. Perhaps one reason for the limited number of previous reports on the pathophysiology of myotonic dystrophy may be due to difficulties in obtaining adequate preparations for study from this patient group.

Up to now, the properties of ionic channels in mammalian muscle have been studied mostly in cultured cells which possess a relatively clean surface membrane suitable for patch clamping (Trautmann, Delaporte & Marty, 1986; Boldin, Jäger,

Ruppersberg & Rüdel, 1987). Burton, Dörstelmann & Hutter (1988) reported the measurement of K^+ and Cl^- channels on excised membrane patches of sarcolemmal vesicles of human muscle fibres. Our approach, using fibre segments of muscle biopsies, may have advantages over those described above, because we record from

Fig. 8. Recording of Na^+ channels in the inside-out mode of patient MyD 14. The membrane was depolarized to the potential indicated. The capacitive artifact was compensated. Up to four channels were activated within a few milliseconds after the beginning of the depolarizing pulse. Late openings can be observed frequently. Low-pass filter at 1·5 kHz.

innervated, adult muscle fibres in which the ionic channels were fully differentiated. Interestingly, aneurally grown cultures of skeletal muscle of patients are in many cases different from adult cells. For example, cells cultured from patients with myotonic dystrophy elicited no spontaneous electrical activity (Merickel, Gray, Chauvin & Appel, 1981; Tamoush, Askanas, Nelson & Engel, 1983; Rüdel *et al.* 1989). This may imply that a functioning neuromuscular contact may be necessary for the expression of myotonia.

Pathophysiology of myotonic dystrophy

Electrical after-activity and slowed relaxation, as recorded both *in vivo* and *in vivo*, were found to be highly correlated. This finding was similar to that reported for recessive generalized myotonia and chondrodystrophia myotonica (Schwartz–Jampel syndrome, SJS) (Iaizzo & Lehmann-Horn, 1989; Lehmann-Horn *et al.* 1989).

It was previously considered that the primary alteration within a sarcolemma which leads to myotonia was a decreased Cl^- conductance (Bryant & Morales-Aguilera, 1971; Bryant, 1973; Furman & Barchi, 1978; Rüdel & Lehmann-Horn, 1985). However, in several patients with myotonic dystrophy whom we investigated, electrical myotonia was found in fibre segments with a normal Cl^- conductance. Thus, an alteration of the Na^+ channel alone may account for this activity.

Similarly, we previously reported that in SJS the hyperexcitability could not be explained by a reduced Cl^- conductance alone (Lehmann-Horn *et al.* 1989). In some fibres (either intact or fibre segments) the Cl^- conductance was normal, but we observed numerous spontaneous bursts of action potentials. The abnormalities observed in the fibres obtained from this patient with SJS were more pronounced, but they were similar to those observed for myotonic dystrophy. In contrast, the myotonic runs which have been recorded from fibres obtained from patients with recessive generalized myotonia have different features (Rüdel & Lehmann-Horn, 1985). Furthermore, all fibre segments from patients with recessive generalized myotonia had both a reduced Cl^- conductance and an altered Na^+ activity (Franke, Iaizzo, Hatt, Ricker & Lehmann-Horn, 1989). Therefore, a number of pathomechanisms, including alteration of Na^+ and Cl^- channels, may lead to electrical hyperexcitability in the myotonic disorders.

Normal and reduced Cl^- conductances of fibres of patients with myotonic dystrophy were reported earlier (Lipicky, 1977), but a systematic investigation of Na^+ currents on adult fibres of patients suffering from myotonic dystrophy was not previously described. Bryant & DeCoursey (1980) used the Vaseline-gap clamp to study Na^+ currents in fibres of myotonic goats. They found that inactivation was slower in myotonic than in normal muscle. According to a mathematical model a decreased rate of Na^+ channel inactivation could enhance myotonia (Bryant, 1982).

The re-opening of Na^+ channels becomes more probable when the membrane is slightly depolarized. If enough channels open spontaneously, local potential changes may occur. Thus, these openings of Na^+ channels probably produced the local potentials which we recorded intracellularly when the membrane was slightly depolarized by the repetitive activity. If the membrane was further depolarized, the majority of the Na^+ channels were inactivated, and action potentials were no longer propagated. In addition, in some fibres we noted that more than one region of a fibre produced local potentials which had different fixed frequencies, because several potential peaks were observed.

Properties of Na^+ channels

For frog skeletal muscle, Patlak & Ortiz (1986) reported late channel openings producing a current with an amplitude of 0·12% of the peak current. In general, the rate of late openings could not be detected in the macroscopic current, because the corresponding time constant of decay was very large and its amplitude was small. Various methods are available to detect this current component. In the past, Kohlhardt, Fröbe & Herzig (1987) pooled data from different patches of myocytes with late Na^+ channel openings and averaged them. In these averages, they found the long component of I_{Na}.

The abnormal behaviour of the Na^+ channels was not caused by the treatment of

the muscle fibre with proteolytic enzymes which are contained in the collagenase, because (i) the control muscles were treated identically and (ii) pronase-modified channels had longer open times than those in control patches (Carbone & Lux, 1986).

It has been reported that spontaneous runs of action potentials which occur in denervated muscle may be due in part to changes in the mechanisms that control the refractory period of Na^+ channels (Kirsch & Anderson, 1986). The alterations of Na^+ channels found in our study are in some respects similar to those found for such denervated muscle (Kirsch & Anderson, 1986). Therefore, it cannot be ruled out that alterations in the ability of Na^+ channels to inactivate as in myotonic dystrophy and SJS are due in part to abnormalities of the neuromuscular junction (McDermot, 1961; Cöers, Telerman-Toppet & Gerard, 1973). Nevertheless, we did not observe TTX-insensitive action potentials in these myotonic muscles which have been described for denervated muscle (Kirsch & Anderson, 1986). Furthermore, in several fibre segments obtained from patients, we noted miniature endplate potentials with normal amplitudes (Ch. Franke, H. Hatt, P. A. Iaizzo & F. Lehmann-Horn, unpublished observation).

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