Electromyography Guides Toward Subgroups of Mutations in Muscle Channelopathies

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Myotonic syndromes and periodic paralyses are rare disorders of skeletal muscle characterized mainly by muscle stiffness or episodic attacks of weakness. Familial forms are caused by mutations in genes coding for skeletal muscle voltage-gated ion channels. Exercise is known to trigger, aggravate, or relieve the symptoms. Therefore, exercise can be used as a functional test in electromyography to improve the diagnosis of these muscle disorders. Abnormal changes in the compound muscle action potential can be disclosed using different exercise tests. We report the outcome of an inclusive electromyographic survey of a large population of patients with identified ion channel gene defects. Standardized protocols comprising short and long exercise tests were applied on 41 unaffected control subjects and on 51 case patients with chloride, sodium, or calcium channel mutations known to cause myotonia or periodic paralysis. These tests disclosed significant changes of compound muscle action potential, which generally matched the clinical symptoms. Combining the responses to the different tests defined five electromyographic patterns (I–V) that correlated with subgroups of mutations and may be used in clinical practice as guides for molecular diagnosis. We hypothesize that mutations are segregated into the different electromyographic patterns according to the underlying pathophysiological mechanisms.

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Familial periodic paralyses and nondystrophic myotonias are disorders of skeletal muscle excitability caused by mutations in genes coding for voltage-gated ion channels. These diseases are characterized by episodic failure of motor activity due to muscle weakness (paralysis) or stiffness (myotonia). Clinical studies have identified three distinct forms of myotonias: myotonia congenita (MC), paramyotonia congenita (PC), and potassium-aggravated myotonia (PAM); and two forms of periodic paralyses: hyperkalemic (hyperPP) and hypokalemic (hypoPP) periodic paralyses, based on changes in blood potassium levels during the attacks.[1–3] MC is caused by mutations in the chloride channel gene *(CLCN1)*, whereas PC and PAM have been linked to missense mutations in the *SCN4A* gene, which encodes the α subunit of the voltage-gated sodium channel.[2,3] To date, two genes have been unquestionably implicated in periodic paralyses, *SCN4A* and *CACNA1S*.[4] The latter encodes the α subunit of the L-type calcium channel, also known as the dihydropyridine receptor. Different missense mutations in the sodium channel gene *(SCN4A)* have been identified in hyperPP patients and a small group of hypoPP

patients (10%) referred to as hypoPP-2.[5] Most hypoPP cases (70%), referred to as hypoPP-1, carry mutations in the *CACNA1S* calcium channel gene. The molecular diagnosis for the remaining 20% has not been established yet. Other forms of skeletal muscle channelopathy, such as myotonic dystrophy, thyrotoxic periodic paralysis, and Andersen–Tawil syndrome, were not addressed in this study.

Ion channels are integral membrane proteins that regulate transmembrane ion fluxes. Skeletal muscle sodium and calcium channels are made of a major poreforming α subunit and smaller auxiliary subunits. Sodium channels are key players for membrane excitability, whereas calcium channels couple membrane excitation to muscle contraction. Chloride channels belong to a different gene family. They play an important role in stabilizing the resting membrane potential and helping membrane repolarization after excitation.

The functional consequences of ion channel mutations on muscle membrane excitability can be studied by electromyography (EMG) in patients. Since weakness may be triggered by strenuous exercise, the use of

strong and sustained voluntary contraction has been proposed as a provocative test for diagnosis. Surface-recorded muscle responses to supramaximal nerve stimulation are used to monitor sarcolemma activity. Analysis of the compound muscle action potential (CMAP) amplitude before and at various times after short (10 seconds) or long (5 minutes) exercise provides information on changes in the number of active fibers and on their ability to depolarize and repolarize. A significant decrease in the CMAP amplitude after a long-exercise test has been reported in approximately 70–80% of the patients with periodic paralyses[6–8] and in 17 and 33% of the patients with MC and PC, respectively.[8] An unexpected observation was the occurrence of a transient paresia in myotonic syndromes after short exercise.[9,10] In the previous EMG studies, patients were grouped only according to the clinical syndromes, with no indication of the causal ion channel mutation. In addition, short and long exercise tests were not systematically used, which turned to be necessary to understand the complex and sometimes puzzling effects of exercise reported by the patients. Indeed, repetition of exercise improves muscle stiffness in MC but not in PC; mild exercise can prevent or delay attacks of weakness, whereas intensive exercise can trigger the attacks in periodic paralyses.

In this study, we explored a group of 51 patients with known ion channel mutations associated with the different forms of periodic paralysis or myotonia. To our knowledge, this work represents the first electromyographic survey on a large number of patients with identified skeletal muscle channelopathies. Inclusive EMG allowed us to establish consistent links between the clinical syndromes and the muscle electrical response to different provocative tests (repeated short ex-

ercise, long exercise). In addition, statistical analysis of the results obtained from several patients carrying the same mutation provided evidence for the EMG changes caused by specific ion channel mutations. Overall, our results suggest that inclusive EMG may guide toward specific ion channel genes and be used as a predictive tool by clinicians who cannot gain easy access to genetic screening.

Patients and Methods

Case Patients and Control Subjects
Symptomatic patients with well-characterized clinical phenotypes[1–3] and identified chloride, sodium, and calcium channel mutations[5] were included in this study.

For the chloride channel, it is now well established that most mutations often concern only one individual.[3] Eighteen patients with a clear MC phenotype were explored. The nature of the chloride channel mutation involved has been identified in 6 of the 18 patients. These six patients were included in the study as a distinct group (Table 1).

For sodium and calcium channels, several mutations are recurrent in the French population, and hence the number of patients carrying a given mutation was large enough to enable statistical comparison between different mutations (see Table 1). We included 24 patients who carried one of the four most encountered sodium channel mutations associated with myotonia (T1313M, R1448C, G1306A, I693T). Although all these patients reported muscle stiffness aggravated by cold, there were some clinical differences between different mutations. Overall, the genotype–phenotype correlations were in line with those previously reported.[1–3] In the most frequent mutations responsible for PC (T1313M and R1448C), patients complained of muscle weakness induced by cold and exercise, with fatigue and difficulty to sustain or repeat exercises. Patients carrying the G1306A mutation reported no weakness but constant and painful muscle stiff-

Table 1. Characteristics of Case Patients and Control Subjects

Clinical Phenotype	Gene	Mutation	Number of Subjects			Age (yr)		No. of Families
			Total	Women	Men	Mean	Range	
Controls			41	22	19	35	12–75	
Myotonia congenita	CLCN1	a	6	2	4	35	20–55	6
Paramyotonia congenita	SCN4A	T1313M	11	7	4	28	6–52	3
	SCN4A	R1448C	5	3	2	27	13–41	4
Potassium aggravated myotonia	SCN4A	G1306A	2	1	1	49	36–62	1
Myotonia + PP	SCN4A	I693T	6	1	5	40	16–52	2
HyperPP	SCN4A	T704M	6	3	3	34	14–51	2
HypoPP-1	CACNA1S	R528H	13	4	9	32	10–54	6
HypoPP-2	SCN4A	R672G-R672H	2	1	1	47	42–52	2
Total (patients)			51	22	29	33	6–62	26

aPatient 1: ms A313T +/− (dominant); Patient 2: ms F167L +/−, ms C277R +/− (recessive); Patient 3: ass 434-2 A > G +/−, dss 1471 + 1 G > A +/− (recessive); Patient 4: ms F306L +/− (dominant or recessive); Patient 5: dss 1471 + 1 G > A +/+ (recessive); Patient 6: ns Q74X +/−, ns R894X +/− (recessive).

+/− = heterozygous; +/+ = homozygous; ms = missense mutation; ass = acceptor splice site mutation; dss = donor splice site mutation; ns = nonsense mutation; coordinates of intronic splice site mutations are given relatively to the numbering of the last nucleotide of preceding exon (donor splice site mutations) or the first nucleotide of following exon (acceptor splice site mutations). Note that for Patient 4, the inheritance pattern is not yet determined and could involve an additional mutation.

ness, occasionally worsened by rest after exercise. This phenotype has been referred to as PAM or sodium channel myotonia.[12] Patients with the I693T mutation experienced both muscle stiffness and episodic weakness, leading to an overlap syndrome of myotonia and hyperPP.[12]

We also explored 6 hyperPP patients with the T704M sodium channel mutation and 13 hypoPP1 patients with the R528H calcium channel mutation. The phenotypes displayed by the patients were similar to those already described.[2–4] Finally, two patients with either the R672G or the R672H mutation of the sodium channel causing hypoPP-2 were also included in this study (see Table 1).

Altogether, a total of 51 case patients (24 with myotonic syndromes, 21 with periodic paralyses, 6 with an overlap syndrome) and 41 control subjects participated in the study, which was conducted after obtaining written informed consent from each individual according to the European Union and French bioethics laws, as well as the Convention of Helsinki.

Electromyography Procedure
Case patients and control subjects were examined using a standardized EMG protocol.[13] CMAPs were evoked by supramaximal nerve stimulation and recorded using skin electrodes. Electrical responses were recorded from right and left abductor digiti minimi (ADM) muscles after stimulation of the ulnar nerves at the wrist, and from the right extensor digitorum brevis (EDB) muscle after stimulation of the anterior tibial nerve at the ankle. Recording electrodes consisted of a pair of small discs carefully positioned to ensure maximal CMAP amplitude. Supramaximal stimulation (single stimulation of 0.3 milliseconds, and 20–30% greater intensity than that needed for maximal CMAP amplitude) of the appropriate nerve was obtained using a bipolar bar electrode held in place manually. Skin temperature was regularly measured and maintained between 32 and 34°C throughout the EMG session, thereby preventing any decrease in CMAP amplitude and area by muscle warming. A bandage around the extreme parts of the recorded muscles prevented articulation displacements and changes in muscle volume during the exercise tests.

CMAPs were first monitored before exercise every 10 seconds for 1–2 minutes to enable baseline stabilization. Neuromuscular transmission was tested by applying repetitive nerve stimulation (10 stimuli at 3Hz). The patient was then asked to contract the muscle as strongly as possible in isometric conditions. After completion of the exercise, the patient was instructed to completely relax while CMAPs were measured at regular time intervals after the end of exercise. Two kinds of exercises were performed. The first type was a short exercise test lasting 10–12 seconds, equivalent to the short exercise test described by Streib and colleagues.[9] CMAPs were recorded 2 seconds immediately after the end of exercise and then every 10 seconds for 50 seconds. The short exercise test was repeated three times with 60 seconds between the beginning of two trials. The second test was one of long exercise lasting 5 minutes with brief (3–4 seconds) resting periods every 30–45 seconds to prevent ischemia. This test is equivalent to the long exercise test described by McManis and colleagues.[6] CMAPs were recorded 2 seconds

immediately after cessation of exercise and then every minute for 5 minutes, and finally every 5 minutes for 40–45 minutes. If the response changed, it was carefully checked that the electrodes had not moved and that stimulation of the nerve remained supramaximal.

The procedure began with a long exercise test of the right ADM muscle, and then a series of three short exercise tests was sequentially performed on the left ADM and the EDB muscle. In some patients, neuromuscular transmission was tested immediately after short exercise by replacing the single stimulus with a repetitive nerve stimulation. Myotonic discharges were also searched using needle recording from several muscles (deltoid, extensor digitorum communis, first interosseus dorsalis, vastus medialis, and tibialis anterior).

Statistical Analysis
CMAP amplitude (peak to peak), total duration, and total area were expressed as a percentage of the reference values measured before exercise. Values plotted on the figures and given in the text are means ± standard errors of the means (SEM). The reference range was defined as the mean ± 2 standard deviations, rounded to the uppermost values for more safety. Outside this range, values were considered abnormal. Both the mean values obtained from all patients with the same mutation and the relative number of patients with abnormal values will be provided. Paired t tests were used to assess the statistical significance of changes induced by exercise in control subjects. The unpaired t test was used to compare one group of patients with the group of control subjects. Because of the relatively small number of patients in each group, the nonparametric Kruskall–Wallis test was used to compare different groups of patients. Statistical significance was quoted as mild ($p < 0.05$), intermediate ($p < 0.01$), or high ($p < 0.001$).

Results
Search for Myotonic Discharges with Needle Electromyography
Myotonic discharges were detected spontaneously with needle EMG in all tested muscles of patients with chloride channelopathies and the following sodium channelopathies: T1313M, R1448C, G1306A, or I693T. There was no difference in their abundance among the different channel mutations. A few short-duration myotonic discharges were also recorded in 50% of hyperPP patients with the T704M sodium channel mutation. These myotonic discharges did not occur spontaneously and were triggered by muscle percussion. In hypoPP-1 and hypoPP-2 patients, myotonic discharges were never found.

Short Exercise Induces Postexercise Myotonic Potentials and Changes of Compound Muscle Action Potential Amplitude in Some Groups of Mutations
In control subjects, short duration of exercise induced a very slight and transient increase of CMAP amplitude in the ADM muscle (5% ± 1%; $p < 0.001$), without any change in shape and duration of the re-

sponse (Fig 1A). The amplitude returned to the pre-exercise value within 10 seconds. Changes in CMAP amplitude between −10 and +20% of the pre-exercise value were considered normal. Note that control subjects ranged from −6 to +14%. No change of CMAP was observed in the EDB muscle.

One of the most striking observations in PC, PAM, and MC patients was the occurrence of surface-recorded repetitive activity immediately after exercise in both the ADM and EDB muscles. The first component of the CMAP response evoked by a single supramaximal stimulus was followed by several signals of decreasing amplitudes, which occurred every 7–10 milliseconds (see Fig 1B, C). Evidence of muscle stiffness could be seen at the same time by muscle palpation. These abnormal responses (postexercise myotonic potentials [PEMPs]) disappeared within 10–30 milliseconds after the completion of exercise. Repetitive 3Hz stimulation performed immediately after exercise cessation provided an additional discriminating criterion between PEMPs and the repetitive discharges caused by synaptic transmission disorders. In most cases, PEMPs were evoked by every stimulus of the train (see Fig 1D), as opposed to the known decline of

repetitive discharges following 3Hz stimulation in myasthenic syndromes. Exceptionally, in one patient with the R1448C sodium channel mutation, repetitive stimulation both abolished PEMP and induced a drastic reduction of the CMAP amplitude, leading to a pseudomyasthenic pattern (Fig 2).

Interestingly, PEMPs were observed in all patients with the T1313M or R1448C (PC) mutation, in one of six patients with the I693T mutation, and in none with the G1306A mutation (PAM). These myotonic responses were also present in one-third of MC patients with known chloride channel mutations but were never recorded from patients with either form of periodic paralysis. The total duration of CMAPs after exercise was measured and compared with the pre-exercise value to assess the importance of the PEMPs. Duration was increased by +123% ± 11% in T1313M and R1448C patients and by only +43% ± 23% in MC patients.

In MC patients with chloride channelopathies, we observed a significant decrease of CMAP amplitude immediately after exercise in both the ADM and EDB muscles (−47% ± 11%; $p < 0.001$; and −54% ± 7%; $p < 0.001$, respectively) (Fig 3C). An
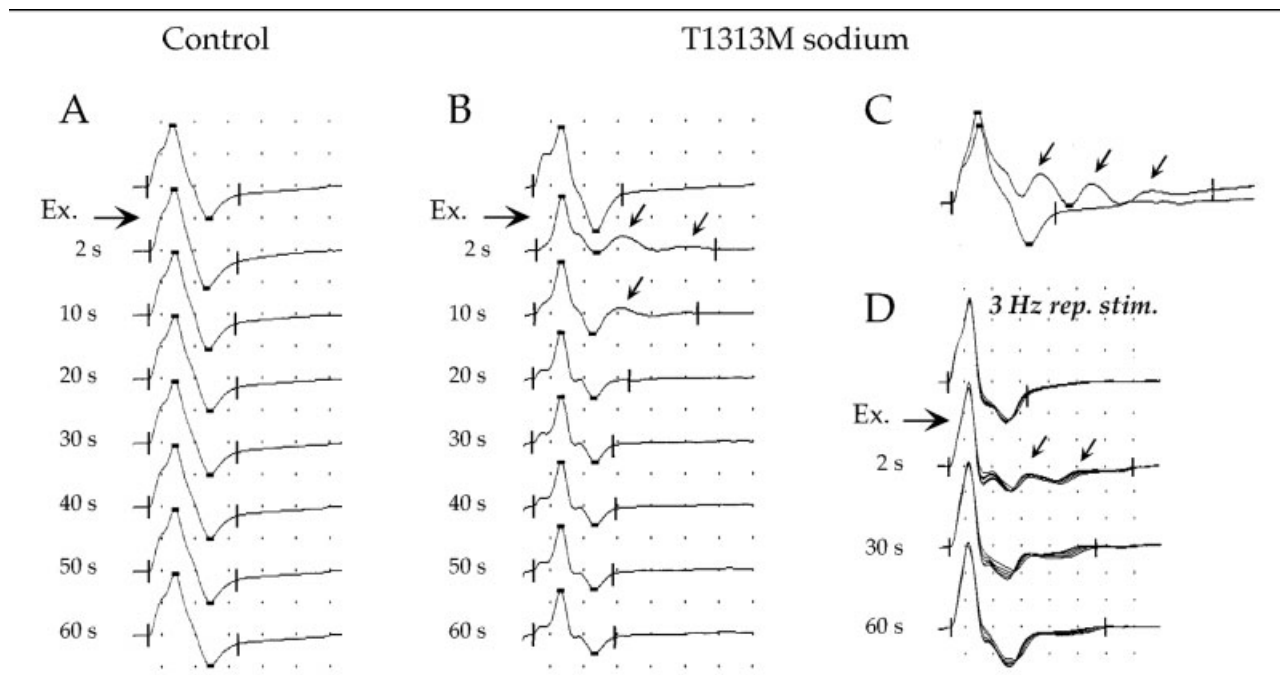
Fig 1. Short exercise test in control subjects and in paramyotonia congenita (PC) patients carrying the T1313M sodium channel mutation. (Top traces) Pre-exercise recording of the abductor digiti minimi compound muscle action potential (CMAP) following ulnar nerve stimulation at the wrist. (Bottom traces) Postexercise recordings at different times after completion of the 10-seconds muscle exercise (Ex.) as indicated to the left of the tracings. (A) Increase (+6%) of CMAP amplitude in an unaffected control. (B) Representative postexercise myotonic potentials (PEMPs) (arrows indicate extra potentials) and 46% decrease of CMAP amplitude induced by T1313M mutation. (C) Superimposed pre-exercise and postexercise CMAP responses to single nerve stimulations in the same patient. (D) Repetitive stimulation of the ulnar nerve (five stimuli at 3Hz) in another T1313M patient. Each tracing represents the five superimposed responses obtained before and after short exercise. Note that PEMPs persisted during the 3Hz stimulation performed immediately after exercise. Scale between two dots: 5 milliseconds, 5mV.

Before exercise

A
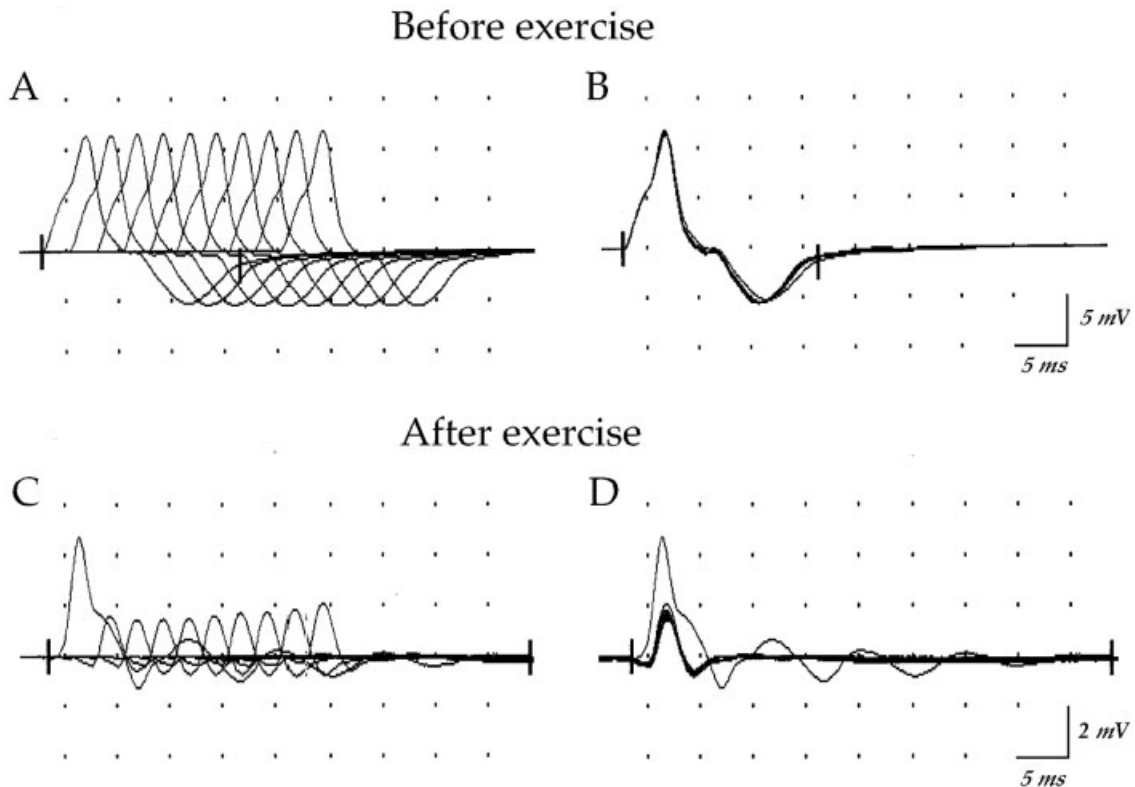
B

5 mV

5 ms

After exercise

C

D

2 mV

5 ms

Fig 2. Repetitive nerve stimulation (10 stimuli at 3Hz) and short exercise test in a paramyotonia congenita (PC) patient carrying the R1448C sodium channel mutation. Sequential (A) and superimposed (B) traces recorded before exercise. (C, D) Similar recordings 2 seconds after short exercise. Note the change in scale between top and bottom tracings. The amplitude of the first response was reduced by 74% compared with the pre-exercise value. Note the decrease in compound muscle action potential (CMAP) amplitude response during the train, with a 63% reduction between the first and the second response. Note also the concomitant disappearance of postexercise myotonic potentials (PEMPs) after the second stimulation.

abnormal decrease (ranging from -17 to -90%) was observed in 83% of these patients. Amplitudes returned to normal values within 20–40 seconds after exercise cessation. For PC patients with T1313M and R1448C sodium channel mutations, the decrease in amplitude ($-36\% \pm 6\%$; $p < 0.001$) was generally less important but persisted at least for 1 minute (see Fig 3D). Only 55% of these patients displayed clearly abnormal values. Such a block of muscle excitability was not observed in most of the patients with G1306A and I693T sodium channelopathies (see Fig 3E). Only two of the eight patients with these mutations showed a postexercise decline outside the reference range.

In hyperPP patients carrying the T704M mutation, exercise of short duration induced an increase of CMAP amplitude ($+23\% \pm 3\%$; $p < 0.001$) that persisted for at least 1 minute. In 83% of hyperPP patients, this increase was significantly higher and lasted longer than that observed in unaffected individuals (Fig 4B). In hypoPP-1 and hypoPP-2 patients, the postexercise increase in CMAP amplitude was not signifi-

cantly different from that observed in control subjects (data not shown).

Repetition of Short-Duration Exercise Aggravates or Suppresses Compound Muscle Action Potential Changes Depending on the Group of Mutations Involved

When repeated, short exercise amplified the increase in CMAP amplitude for hyperPP patients carrying the T704M sodium channel mutation ($+64\% \pm 11\%$; $p < 0.001$ after the third trial) (see Fig 4B). This observation does not hold for hypoPP-1 patients. For MC patients, the postexercise decrease in CMAP amplitude disappeared with repetitive trials (see Fig 4C). This effect was also observed in patients with G1306A and I693T mutations, who displayed a decline in amplitude after the first exercise. In contrast, there was a drastic worsening for PC patients with T1313M and R1448C sodium channel mutations. All of these patients (including those who did not show any CMAP decline after the first trial) displayed a marked reduction of CMAP amplitude after the third trial (-58%
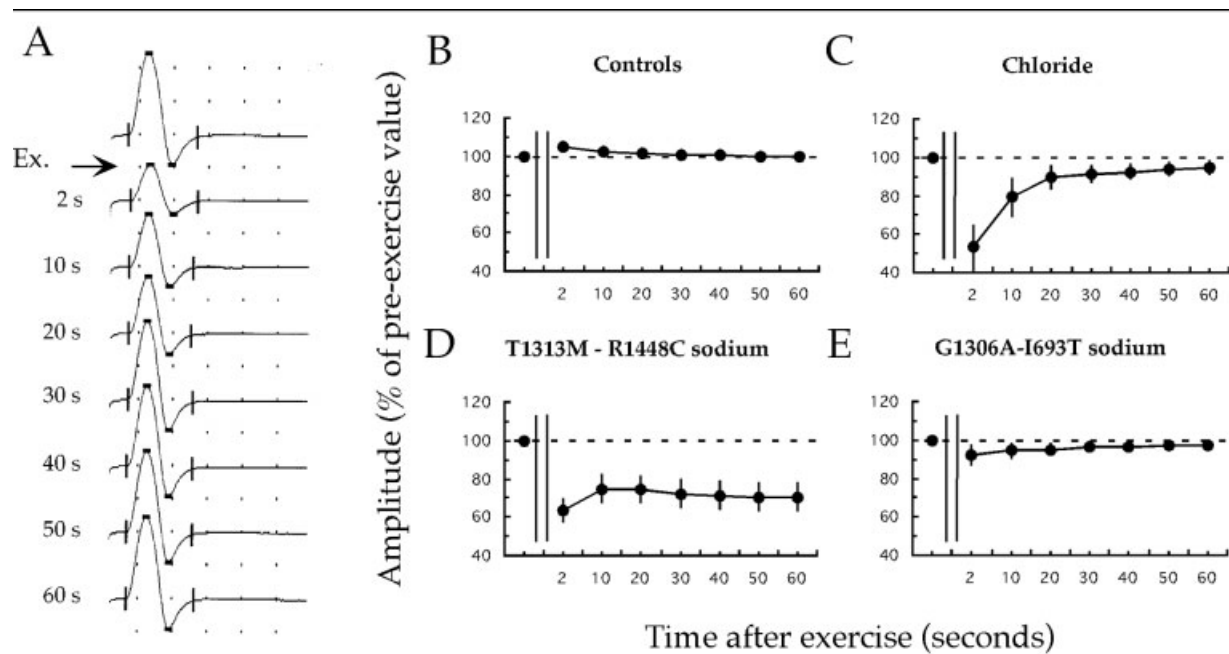
Fig 3. Short exercise test in myotonic syndromes. (A) Transient decrease of compound muscle action potential (CMAP) amplitude (−55%) after short exercise in a myotonia congenita (MC) patient with the F167L–C277R chloride channel mutation. Pre-exercise (top trace) and postexercise recordings (bottom trace) at different times after the trial (Ex.) as indicated to the left of the tracings. Scale between two dots: 5 milliseconds, 5mV. (B–E) Changes in CMAP amplitude of abductor digiti minimi (ADM) muscle following short exercise (double bars) in 41 unaffected controls (B), 6 MC patients with chloride channel mutations (C), 16 paramyotonia congenita (PC) patients with T1313M or R1448C sodium channel mutations (D), and 8 patients with G1306A or I693T sodium channel mutations (E). The amplitude of the CMAP expressed as a percentage of its pre-exercise value is plotted against the time elapsed after the exercise trial (symbols and vertical bars). Means ± standard errors of the means.

± 8%; $p < 0.001$), up to −92% in some cases (see Fig 4D). In addition to CMAP declines, PEMPs recorded after the first trial in T1313M and R1448C patients gradually decreased in later trials until total disappearance. Similar results were obtained from the ADM and EDB muscles.

Long Exercise Test Discloses Further Changes of Compound Muscle Action Potentials
In control subjects, a long exercise test slightly decreased CMAP amplitude (−6% ± 1%; $p < 0.001$) and greatly increased duration (+38% ± 4%; $p < 0.001$), which resulted in an increase of the total CMAP area (+24% ± 3%; $p < 0.001$) immediately after exercise completion. Recovery of pre-exercise values occurred after 30–60 seconds and remained unchanged within the following 40–50 minutes (provided the skin temperature was constantly maintained) (Fig 5B). Changes in CMAP amplitude between −20 and +10% of the pre-exercise value were considered normal. Control subjects ranged from −16 to +5%.

In MC patients, a slight and transient decrease of CMAP amplitude (−13% ± 4%) was evidenced, but it was not significantly different from that observed in control subjects (see Fig 5C). The decrease was outside the reference range only in one-third of these patients. In contrast, all PC patients with T1313M and R1448C sodium channel mutations showed an immediate, severe, and persistent impairment of muscle excitability (decrease of CMAP amplitude −66% ± 6%; $p < 0.001$), which lasted at least 30–40 minutes (see Fig 5D). Because of the occurrence of a profound weakness during the exercise, two subjects with the R1448C mutation were unable to prolong the exercise beyond 1 minute. In these cases, the decrease in CMAP amplitude reached up to −95%. This finding was never observed in patients with G1306A sodium channel mutations (see Fig 5E).

In patients with periodic paralysis, CMAP changes occurred either immediately after exercise cessation or later (Fig 6). Within the first seconds after exercise, a slight and transient increase of CMAP amplitude was observed in hyperPP patients with T704M sodium channel mutations (+13% ± 5%; $p < 0.001$) but not in hypoPP-1 patients, with the exception of one (see Fig 6). Then, 10–20 minutes after the end of exercise, a significant and prolonged decrease of CMAP amplitude and area appeared at rest in hyperPP and hypoPP-1 patients (−51% ± 10%; $p < 0.001$ and −54% ± 5%; $p < 0.001$, respectively). The decline was up to −93%. Note that 1 of 13 patients with hypoPP-1 did not display any change in the response.
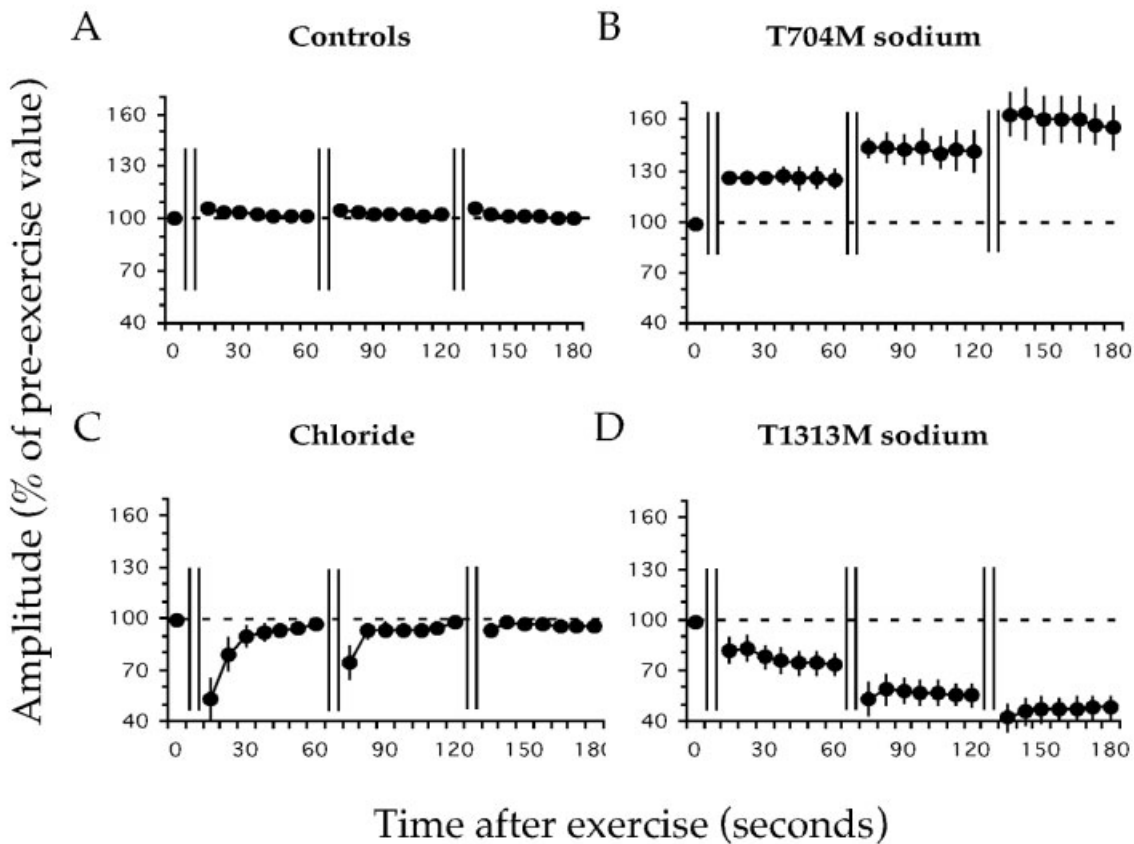
Fig 4. Effects of short exercise repetition in myotonic syndromes and periodic paralysis. Short exercise of the abductor digiti minimi (ADM) muscle was repeated three times successively at 1-minute intervals. (double bars) Successive trials. Compound muscle action potentials (CMAPs) were recorded during the 50-seconds resting periods. The amplitude of the CMAP, expressed as a percentage of its value before the trials, is plotted against the time elapsed after the first exercise trial in unaffected controls (A), 6 hyperPP patients with T704M sodium channel mutations (B), 6 myotonia congenita (MC) patients with chloride channel mutations (C), and 11 paramyotonia congenita (PC) patients with T1313M sodium channel mutation (D) (symbols and vertical bars). Means ± standard errors of the means.

The late decrease was significantly milder in hypoPP-2 patients (−23% ± 6%; p < 0.001).

In hyperPP patients, a short exercise trial during the paretic phase induced a marked CMAP increment (+78% ± 21%; p < 0.001), partially correcting the loss of excitability. In one hypoPP-1 patient, the decrease in CMAP amplitude of the exercised ADM muscle appeared immediately after exercise completion and was associated with a severe and persistent drop of the electrical response recorded in nonexercised contralateral ADM as well as EDM muscles. A paralytic attack was clinically evident at the same time.

Electromyographic Outcome in Myotonias and Periodic Paralysis Discloses Five Different Patterns
By comparing EMG findings and responses to short and long exercise tests, most patients carrying the same mutation shared a similar pattern of muscle electrical abnormalities. Five main electrophysiological patterns

could be defined, as specified in Table 2, each of them corresponding to a defined group of mutations.

Patients with myotonia could be separated in three groups (patterns I–III). PC patients with T1313M or R1448C mutations exhibited myotonia and PEMPs with postexercise decrease in CMAP amplitude that worsened with repeating or prolonging exercise (pattern I). Regardless of the nature and localization of the causal mutation, most of MC patients with chloride channelopathy displayed similar electrophysiological changes characterized by myotonia with transient decreased CMAP amplitude after short exercise that disappeared with repeating or prolonging exercise (pattern II). In most patients with G1306A or I693T sodium mutations, myotonia was not associated with postexercise CMAP changes (pattern III). HyperPP patients with the T704M sodium mutation showed another pattern (pattern IV) characterized by immediate increase and delayed decrease of CMAP amplitude after exercise. HypoPP patients with the R528H calcium
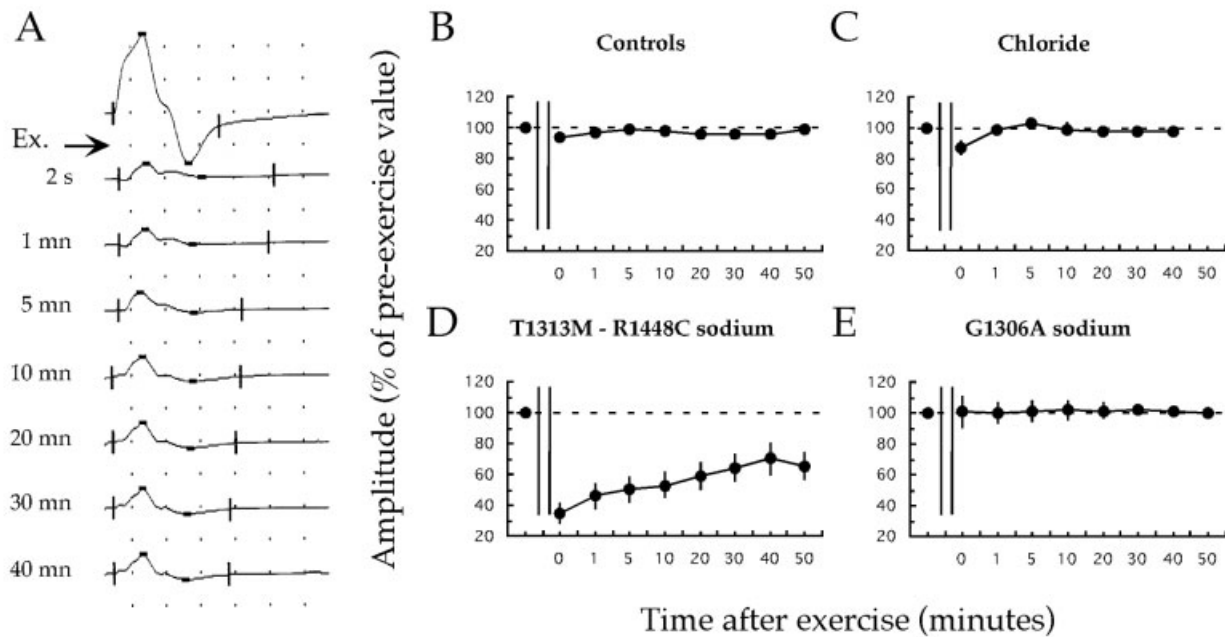
Fig 5. Long exercise test in myotonic syndromes. (A) Immediate and persistent decrease of compound muscle action potential (CMAP) amplitude (−85%) after long exercise in a paramyotonia congenita (PC) patient with the T1313M sodium channel mutation. Pre-exercise (top trace) and postexercise recordings (bottom trace) at various times following the trial (Ex.) as indicated to the left of the tracings. Scale between two dots: 5 milliseconds, 5mV. (B–E) Changes in CMAP amplitude of the abductor digiti minimi (ADM) muscle after long exercise (double bars) in 41 unaffected controls (B), 6 myotonia congenita (MC) patients with chloride channel mutations (C), 16 PC patients with T1313M or R1448C sodium channel mutations (D), and 2 patients with G1306A sodium channel mutations (E). The amplitude of the CMAP, expressed as a percentage of its pre-exercise value, is plotted against the time elapsed after the exercise trial (symbols and vertical bars). Means ± standard errors of the means.

channel mutation could be individualized by a delayed decrease in CMAP amplitude after long exercise without immediate change after short or long exercise (pattern V). The sensitivity of one pattern for the corresponding group of mutations is given in Table 2.

The correlation between EMG findings and groups of mutations was further analyzed by studying the distribution of electrophysiological phenotypes (Table 3). Pattern I was observed exclusively in the 16 PC patients with the T1313M or R1448C sodium channel mutation. The group of patients with phenotype II was composed mainly of 83% of the patients with chloride channel mutations. It also included two patients with G1306A and I693T sodium channel mutations, respectively. Conversely, the group of patients with pattern III included 63% of the patients with G1306A and I693T sodium channel mutations and one patient with the F306L chloride channel mutation.

Patterns IV and V were composed of 83% (5 of 6) of T704M patients and 84% (11 of 13) of R528H patients, respectively (see Table 3). Each pattern grouping included one patient of the alternative category. The two hypoPP-2 patients with R672 sodium channel mutation displayed phenotypes IV and V, respectively. The normal pattern included all control subjects and one patient with hypoPP-1.

Discussion

We have carried out an extensive EMG study on a large population of patients with identified mutations of voltage-gated skeletal muscle ion channels causing periodic paralyses or myotonia. For the first time, several patients carrying the same mutations were explored using both needle EMG and different provocative exercise tests with surface recordings. Abnormal changes in muscle electrical activity could be correlated with the clinical symptoms. Most interestingly, our results disclosed five distinct patterns (I–V), each of which consistently correlated with a group of patients carrying the same genetic defects.

A Novel Electromyographic Sign of Myotonia: Postexercise Myotonic Potentials

The presence of postexercise myotonic activity in PC patients carrying T1313M or R1448C sodium channel mutation (pattern I) and in MC patients (patterns II) indicates that muscle fibers fire repeatedly after a single nerve stimulation. To date, there are only a few reports of surface-recorded repetitive firing in myotonic disorders.[13,14] Interestingly, these myotonic discharges occurred in all PC patients with T1313M or R1448C mutations and only in one-third of MC patients. Such postexercise surface-recorded myotonic activity was ob-
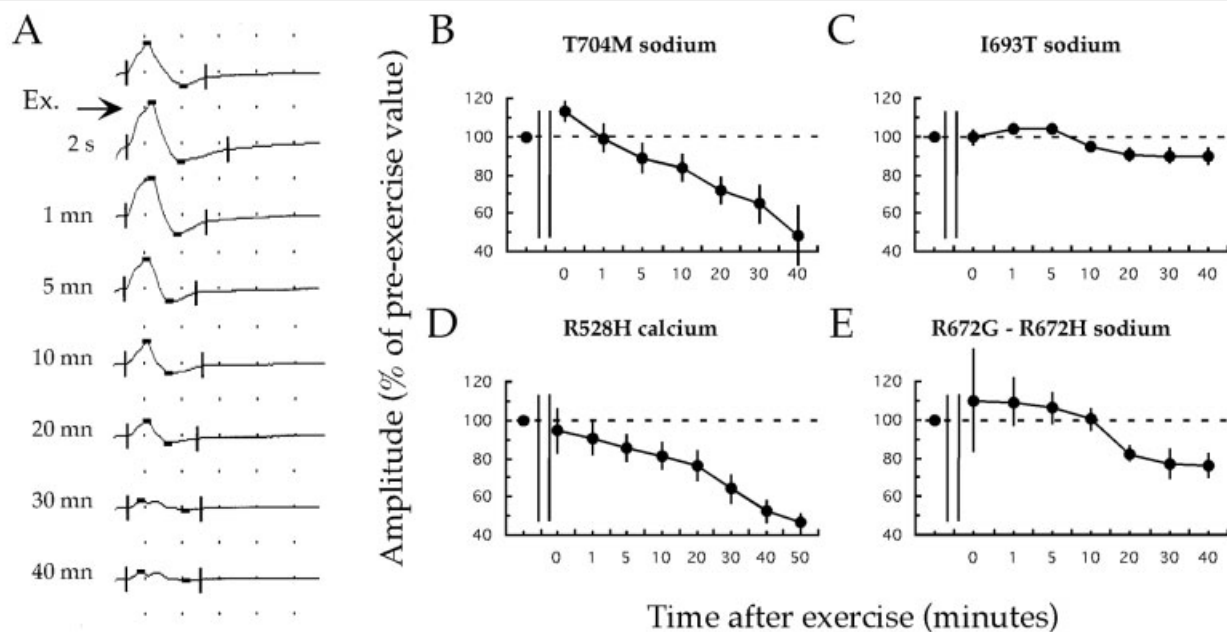
Fig 6. Long exercise test in periodic paralyses. (A) Early increase (+38%) and delayed decrease (−74%) of compound muscle action potential (CMAP) amplitude after long exercise in hyperPP patient with the T704M sodium channel mutation. Pre-exercise (top trace) and postexercise recordings (bottom trace) at different times following the trial (Ex.) as indicated left of the traces. Scale between 2 dots: 5 milliseconds, 5mV. (B–E) Changes in CMAP amplitude of the abductor digiti minimi (ADM) muscle after long exercise (double bars) in 6 hyperPP patients with T704M sodium channel mutations (B), 6 Myotonia-hyperPP patients with the I693T mutation of the sodium channel (C), 13 hypoPP-1 patients with the R528H calcium channel mutation (D), and 2 hypoPP-2 patients with R672G or R672G sodium channel mutations (E). The amplitude of the CMAP, expressed as a percentage of its pre-exercise value, is plotted against the time elapsed after the exercise trial (symbols and vertical bars). Means ± standard errors of the means.

Table 2. Electrophysiological Patterns of Most Frequent Responses to Exercise Tests and EMG Recordings

Clinical phenotype		PC	MC	Other Forms of myotonia	HyperPP	HypoPP-1
Channel mutations		T1313M or R1448C sodium	Chloride	G1306A or 1693T sodium	T704M sodium	R528H calcium
Electrophysiological pattern		I	II	III	IV	V
Needle-EMG	Myotonic discharges	Abundant	Abundant	Abundant	No or rare	No
CMAPs after short exercise	PEMP	Yes	Yes or no	No	No	No
	Amplitude change after first trial	Increase or decrease	Transient decrease	No	Increase	No
	Amplitude change after second or third trial	Gradual decrease	No	No	Gradual increase	No
CMAPs after long exercise	Immediate change of amplitude	Decrease	No or slight decrease	No	Increase	No
	Late change of amplitude	Decrease	No	No	Decrease	Decrease
Sensitivity[a] (%)		100	83	63	83	84

[a]The sensitivity of one pattern for the corresponding group of mutations was defined as the number of patients displaying the pattern versus the total number of patients carrying the same mutation.

EMG = electromyography; PC = paramyotonia congenita; MC = myotonia congenita; pp = periodic paralysis; CMAP = compound muscle action potential; PEMP = postexercise myotonic potential.

Table 3. Distribution of the Electrophysiological Patterns[a] in Groups of Patients Sharing Similar Mutations and Clinical Syndromes

Clinical Phenotype (mutations)	No. of Patients	Electrophysiological Pattern					
		I	II	III	IV	V	Normal[b]
Controls	41	0%	0%	0%	0%	0%	100%
PC (T1313M-R1448C sodium)	16	100%	0%	0%	0%	0%	0%
MC (chloride)	6	0%	83%	17%	0%	0%	0%
PAM (G1306A sodium)	2	0%	50%	50%	0%	0%	0%
Myotonia + PP (I693T sodium)	6	0%	17%	66%	17%[c]	0%	0%
HyperPP (T704M sodium)	6	0%	0%	0%	83%	17%	0%
HypoPP-2 (R672G or H sodium)	2	0%	0%	0%	50%	50%	0%
HypoPP-1 (R528H calcium)	13	0%	0%	0%	8%	84%	8%

[a]As defined in Table 2.
[b]No myotonic discharges with needle electromyography, no abnormal change of compound muscle action potential amplitude or shape after short and long exercises.
[c]One patient with a mixed electrophysiological phenotype: pattern II for short exercise, and pattern IV for long exercise.

PC = paramyotonia congenita; MC = myotonia congenita; PAM = potassium-agravated myotonia; pp = periodic paralysis.

served in only 1 (I693T) of 8 patients with G1306A or I693T sodium channel mutations, although myotonic discharges were easily detected with the needle in all muscles explored at rest (pattern III). This finding suggests that PEMPs occur only when exercise leads to repetitive firing of several muscle fibers in a synchronous manner. If detected in a patient, PEMP should imply the presence of ion channel mutations with type I or type II pattern.

Relation of Postexercise Myotonic Potentials to Myasthenic Syndromes
PEMPs are comparable to the well-known repetitive discharges observed after rest in neuromuscular junction disorders such as acetylcholinesterase deficiency and slow-channel syndrome.[15] In these disorders, repetitive responses have been explained by excess-of-function defects, that is, excess of acetylcholine and impairment of the acetylcholine receptor channel inactivation, respectively. However, in myasthenic syndromes, repetitive responses disappear with voluntary contraction or repetitive stimulation.[16] Conversely, our results show that in myotonic syndromes, repetitive firing was induced by voluntary contraction and was not reduced by 3Hz stimulation.

It should nevertheless be noted that a 3Hz stimulation given after short exercise could induce a decrease of CMAP amplitude and stop the repetitive firing in few T1313M and R1448C PC patients, therefore mimicking a myasthenic syndrome. This finding is reminiscent of the recent description of myasthenic syndrome caused by V1442E sodium channel mutations.[17] In the latter report, a 2Hz stimulation had no effect on CMAPs in rested muscles but induced a 50% decrease after a conditioning train of 10Hz for 1 minute. In vitro data provided arguments in favor of a loss-of-function defect caused by a failure in action potential initiation. In a few T1313M and R1448C myo-

tonic syndromes, postexercise stimulation at 3Hz suppressed the repetitive responses and induced a long-lasting drop in CMAP amplitude, which suggests a gain-of-function defect involving voltage-gated ion channels.[18] A plausible explanation is that 3Hz stimulation aggravated membrane depolarization, thereby bringing many firing fibers into a state of inexcitability.

Electromyography Distinguishes between Different Subgroups of Sodium Channelopathies
The data obtained from myotonic patients carrying sodium channel mutations could be divided into two main patterns (patterns I and III). In pattern I (patients with T1313M or R1448C mutations), both long exercise and repetition of short exercise led to the disappearance of PEMP and ultimately to a long-lasting decrease of the muscle electrical response. This finding was not observed in most patients with pattern III (G1306A or I693T mutations), for whom excitability was not impeded by exercise trials. These EMG outcomes correlate nicely with the symptoms, as patients presenting pattern I displayed exercise-induced episodes of muscle weakness, whereas patients with pattern III did not. We suggest that, in pattern I, repeated short exercise or prolonged exercise induces a sustained membrane depolarization, leading to muscle electrical refractoriness. These EMG patterns are reminiscent of excitability patterns obtained from simulation studies on PC (T1313M) and PAM (G1306E) mutations.[18] In these experiments, simulated muscle spikes predicted myotonic discharges followed by a return to the resting potential for cells containing G1306E and predicted myotonia plus a depolarization block for those with T1313M. This finding was correlated with the presence of abnormally sustained sodium current in T1313M but not in G1306E.[18] In addition, all mutations with pattern I (T1313M, R1448C) induce a marked slowing of channel inactivation,[18,19] whereas in

pattern III mutations, inactivation is either slowed to a lesser extent (G1306A)[18] or not altered (I693T).[20] Overall, both the presence of a sustained current and the degree of slowing of channel inactivation may determine the excitability pattern.

Electromyography Distinguishes between Chloride and Sodium Channelopathies

In agreement with previous reports,[9,10] short exercise after rest induced a transient decline of CMAP amplitude in most myotonic syndromes. Repeating or prolonging exercise reversed this block of muscle excitability in most MC patients with chloride channelopathies (pattern II). Although a decrease in amplitude is a sign of muscle weakness rather than stiffness, it is tempting to correlate exercise-induced recovery of CMAPs with the well-established notion that in MC patients, myotonia is relieved by exercise. The presence of pattern II speaks, therefore, in favor of a chloride channel gene defect. However, pattern II was also found in one of two patients carrying the PAM G1306A sodium channel mutation. Conversely, a few MC patients did not show any change in CMAPs following exercise and belonged to pattern III together with sodium channel mutants. One explanation could be that the decrease in the muscle electrical response is related to the type of chloride channel mutation causing MC, as suggested by a recent study.[21] An alternative hypothesis could be that the presence of MC patients in pattern III simply reflects phenotypic variations with minimal expression of the mutations.

Electromyography Distinguishes between Sodium and Calcium Channel Mutations

Periodic paralysis patients could be divided into two groups (patterns IV and V). The loss of muscle excitability, which occurs at rest following an exercise trial, correlates with the muscle weakness experienced by these patients after strenuous exercise and is a common feature to both patterns. Previous reports have shown that long exercise induces a transient increase prior to the long-lasting decrease of CMAPs in hyperPP.[6,8] Our results show that the increment in CMAPs was present immediately after both short and long exercise and was greater in paretic muscles or when short exercise was repeated (pattern IV). This correlates well with the observation that hyperPP patients declare that repeated mild activity can improve their muscle strength and prevent or delay attacks of paralysis. This may be related to some physiological processes that normally participate in membrane repolarization following exercise, such as an increased activity of the sodium/potassium pump or an increased potassium efflux.

The early incremental effect of repeated short exercise or long exercise on CMAPs was not observed in hypoPP-1 patients (pattern V). In addition, results of needle searches of myotonic discharges were always negative. These results, together with the late decline in CMAP response following exercise, speak in favor of a reduced membrane excitability.[4,22]

Conclusion

Electrophysiological exploration of patients with well-characterized mutations showed different patterns that may be related to distinct pathophysiological mechanisms, enabling discrimination between different forms of periodic paralyses and myotonias. Most of our observations were based on a sufficient number of well-characterized cases for use in clinical practice and may guide molecular diagnosis.

References

1. Ptacek L. The familial periodic paralyses and nondystrophic myotonias. Am J Med 1998;105:58–70.
2. Cannon SC. Ion channel defects in the hereditary myotonias and periodic paralysis. In: Martin JB, ed. Molecular neurology. New York: Scientific American, 1998:257–277.
3. Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. Physiol Rev 1999;79:1317–1372.
4. Cannon SC. An expanding view for the molecular basis of familial periodic paralysis. Neuromuscul Disord 2002;12: 533–543.
5. Sternberg D, Maisonobe T, Jurkat-Rott K, et al. Hypokalaemic periodic paralysis type 2 caused by mutations at codon 672 in the muscle sodium channel gene SCN4A. Brain 2001;124: 1091–1099.
6. McManis PG, Lambert EH, Daube JR. The exercise test in periodic paralysis. Muscle Nerve 1986;9:704–710.
7. Subramony SH, Wee AS. Exercise and rest in hyperkalemic periodic paralysis. Neurology 1986;36:173–177.
8. Kuntzer T, Flocard F, Vial C, et al. Exercise test in muscle channelopathies and other muscle disorders. Muscle Nerve 2000;23:1089–1094.
9. Streib EW, Sun SF, Yarkowski T. Transient paresis in myotonic syndromes: a simplified electrophysiologic approach. Muscle Nerve 1982;5:719–723.
10. Streib EW. Differential diagnosis of myotonic syndromes. Muscle Nerve 1987;10:603–615.
11. Rüdel R, Ricker K, Lehmann-Horn F. Genotype-phenotype correlations in human skeletal muscle sodium channel diseases. Arch Neurol 1993;50:1241–1248.
12. Plassart E, Eymard B, Maurs L, et al. Paramyotonia congenita: genotype to phenotype correlation in two families and report of a new mutation in the sodium channel gene. J Neurol Sci 1996;142:126–133.
13. Fournier E. Tests d'effort. In: Fournier E, ed. Examen électromyographique et étude de la conduction nerveuse, sémiologie électrophysiologique. Cachan: E. M. Inter, 1998:253–262. [In French.]

14. Kim DS, Kim EJ, Jung DS, et al. A Korean family with Arg1448C mutation of SCN4A channel causing paramyotonia congenita: electrophysiologic, histopathologic, and molecular genetic studies. J Korean Med Sci 2002;17:856–860.

15. Engel AG, Ohno K, Sine SM. Congenital myasthenic syndromes. In: Engel AG, ed. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press, 1999:251–97.

16. Harper CM Jr. Electrodiagnosis of endplate disease. In: Engel AG, ed. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press, 1999:65–84.

17. Tsujino A, Maertens C, Ohno K, et al. Myasthenic syndrome caused by mutation of the SCN4A sodium channel. Proc Natl Acad Sci U S A 2003;100:7377–7382.

18. Hayward LJ, Brown RH, Cannon SC. Inactivation defects caused by myotonia-associated mutations in the sodium channel III-IV linker. J Gen Physiol 1996;107:559–576.

19. Yang N, Ji S, Zhou M, et al. Sodium channel mutations in paramyotonia congenita exhibit similar biophysical phenotypes in vitro. Proc Natl Acad Sci U S A 1994;91:12785–12789.

20. Plassart-Schiess E, Lhuillier L, George AL Jr, et al. Functional expression of the Ile693Thr Na+ channel mutation associated with paramyotonia congenita in a human cell line. J Physiol (Lond) 1998;507:721–727.

21. Colding-Jorgensen E, Duno M, Schwartz M, Vissing J. Decrement of compound muscle action potential is related to mutation in myotonia congenita. Muscle Nerve 2003;27:449–455.

22. Ruff RL. Skeletal muscle sodium current is reduced in hypokalemic periodic paralysis. Proc Natl Acad Sci U S A 2000;97:9832–9833.