DIFFERENTIAL DIAGNOSIS OF PERIODIC PARALYSIS AIDED BY *IN VITRO* MYOGRAPHY

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Abstract—*In vitro* twitch tests were performed on excised muscle bundles from 30 periodic paralysis (PP) patients in an attempt to verify the somatic origin of PP, and to differentiate between the hypokalemic (HypoPP) and the hyperkalemic forms (HyperPP). Seventeen PP patients with a definite diagnosis of familial HypoPP, familial HyperPP (subsequently confirmed by SCN4A mutations), or thyrotoxic PP entered the study, as well as 13 patients with a history of attacks of weakness but with negative clinical provocation tests and therefore ambiguous diagnosis; 15 normal subjects served as controls. In contrast to control, bundles from patients with clear diagnosis went into sustained paralysis on exposure to Cl-free solution. Exposure to K^+ channel activators induced a large increase in force. Specifically for HypoPP muscle, low extracellular $[K^+]$ decreased twitch force which was further reduced by addition of insulin or adrenaline, whereas HyperPP bundles responded with an irreversible decrease in twitch force when extracellular $[K^+]$ was elevated. Out of the 13 patients with unclear diagnosis, the *in vitro* studies made it possible to classify 10 as HypoPP and one as HyperPP (later confirmed by a M1592V mutation). In the remaining two patients who claimed to suffer from paralytic attacks, all *in vitro* tests were normal, questioning the occurrence of dyskalemic PP. The results demonstrate that *in vitro* tests can be used to ensure the proper diagnosis to a high percentage when clinical provocative tests have failed.

Key words: Hypokalemic periodic paralysis, hyperkalemic periodic paralysis, thyrotoxic periodic paralysis, *in vitro* contraction test.

INTRODUCTION

Periodic paralysis (PP) is the common symptom for a group of rare diseases of different etiology [1]. Patients suffer from episodes of weakness for which typical triggers would be rest after strenuous work, certain kinds of food, or mental stress. Various origins are known for the condition, e.g. the disease can be inherited or acquired, somatic or psychogenic. Differentiation between these alternatives can be difficult when the family history is inconclusive and provocative clinical tests (an oral K^+ load or a glucose/insulin load) do not provide decisive clues. The choice of the appropriate therapy is then difficult, and it is not uncommon that a patient is treated in a way that slightly aggravates his condition and this fact might go unnoticed for a substantial length of time.

A common classification of PP relates to the change of serum K^+ levels during an attack of paralysis which may fall, stay constant or rise. Accordingly, in many cases clinical differentiation into hypokalemic PP (HypoPP) or hyperkalemic PP (HyperPP) may be achieved simply by measuring serum $[K^+]$ during an attack of weakness. However, quite often serum potassium does not change much during an attack and moreover, even in HyperPP patients this parameter may fall below normal at the end of an attack. This may result in a wrong diagnosis.

For familial HyperPP the situation was improved when molecular biology revealed that the disease is usually caused by some of the several point mutations identified in the SCN4A gene that encodes the adult skeletal

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muscle Na^+ channel α-subunit [2–12]. Thus, for most of these patients indirect and direct genotyping is now possible, although for one HyperPP family [13] linkage to this gene was excluded.

Unfortunately for the much more common form of PP, familial HypoPP, markers for DNA analysis have not been detected earlier than 1994 [14]. The pattern of inheritance in HypoPP is autosomal dominant but often there is incomplete penetrance in females [15].

In our long-standing experience with diagnosing such families, we have come to the conclusion, that whenever the diagnosis on the basis of the usual provocative tests with such patients is ambiguous, the addition of some *in vitro* tests provides more specific criteria [16]. The primary objective of the present investigations was therefore to establish standardized procedures which would allow specialized medical centers to verify and/or determine the specific diagnosis in difficult cases of PP.

PATIENTS

Thirty patients with reported episodic attacks of weakness gave informed consent for a thorough clinical examination. They were chronologically numbered for easier cross-referencing with our other publications. A spontaneous attack was observed with none of them while they were in hospital. No cardiac abnormalities were observed.

The patients were divided into four different groups according to the following diagnostic criteria (Table 1).

(1) *HyperPP*. (i) Autosomal dominant inheritance, (ii) early onset in childhood, (iii) reports of short-lasting attacks of weakness, usually in the morning, during rest after exercise, or when fasting, (iv) normal serum K^+ levels in the attack-free interval, (v) a positive work or K^+ test, i.e. weakness occurring 30-60 min after exercise or oral intake of K^+ (1 mmol/kg body wt). The presence of clinical or electrical myotonia was taken as further evidence for the diagnosis whereas absence of myotonic signs was considered inconclusive. Ten responded to the provocative test.

(2) *HypoPP*. (i) Autosomal dominant inheritance, (ii) onset in the first or second decade of life, (iii) typical reports of attacks of weakness, usually in the second half of the night or in the early morning (after strenuous activity the

preceding evening plus a carbohydrate-rich supper) with strength gradually increasing as the day passes, or during the day, provoked by intake of a diet rich in carbohydrates or Na^+, (iv) normal serum K^+ levels in the attack-free intervals, and (v) a positive glucose/insulin test, i.e. occurrence of weakness 1–2 h after administration of both glucose (orally 3 g/kg or by infusion 3 g/kg lasting 1 h) and insulin (subcutaneously 15–20 IU). Five patients entered this group. Three of them fulfilled all criteria, for the other two low serum $[K^-]$ had been reported during spontaneous attacks.

(3) *Thyrotoxic PP (ThyPP)*. Criterion for this classification was the absence of familial cases and the presence of thyrotoxicosis. Two patients entered this group.

(4) *Ambiguous*. In the remaining group of 13 patients, the diagnosis was initially uncertain as the results of the provocative *in vitro* tests were inconclusive (Table 2). On the other hand, the presence of electrical myotonia in patient J favored the diagnosis HyperPP.

MATERIALS AND METHODS

Small muscle specimens (length > 2.5 cm) were taken from all patients under local anaesthesia or nerve block from either the biceps bracchii or the quadriceps femoris muscles. The biopsies from 15 subjects who were tested for susceptibility to malignant hyperthermia served as controls after susceptibility had been excluded. From each specimen several bundles (diameter 2–3 mm) were prepared for myography [17]. All procedures were in accordance with the Helsinki convention and were approved by the Ethical Committees of the Technical University of Munich and the University of Minnesota.

Solutions

The standard solution used for transportation, dissection, and electrophysiological experiments contained in mmol/l: NaCl, 108; KCl, 3.5; $CaCl_2$, 1.5; $MgSO_4$, 0.7; $NaHCO_3$, 26.2; NaH_2PO_4, 1.7; Na gluconate, 9.6; glucose, 5.5; sucrose, 7.6 (total osmolarity 317 mosmol/l). For the bundles obtained from the HypoPP patients a slightly higher K^+ concentration (4.5 mM vs 3.5 mM) was used in the standard solution in order to avoid the induction of weakness.

Table 1. Clinical information about patients with a clear diagnosis prior to *in vitro* studies.

	Age and sex	Family history	Onset age	Frequency of attacks	Duration of attacks	Other symptoms	Permanent weakness	Clinical provocation	Genetic confirm.
HypoPP4	29 m	+	16	1 per month to 2 per week	3–4 h	Fluctuating weakness	None	(+)	–
HypoPP8	24 m	+	4	2 per week	2 h–1 day	None	Moderate	[K⁺]* = 2.5 mM	–
HypoPP9	25 f	–	9	1 per month	Days	None	None	(+) Severe weakness	–
HypoPP15	60 m	+	15	1 per week	Hours	None	Moderate	(+)	–
HypoPP16	24 m	+	13	2 per month	1/2–2 days	None	None	[K⁺]* = 2.5 mM	–
HyperPP1	27 f	+	12	1 per month	1/2–2 h	Myotonia	None	(+) Gen. weakness	M 1592 V
HyperPP2	56 f	+	6	1 per month	Days	None	Slight	(+) Gen. paralysis	T 704 M
HyperPP3	28 f	+	12	1 per month	3 days	Myotonia	None	(+) Severe weakness	M 1592 V
HyperPP4	37 m	+	8	1 per month	Days	Atrophy	Moderate	(+) Gen. paralysis	T 704 M
HyperPP5	24 m	+	10	Daily	1/2–2 h	None	None	(+) Severe weakness	T 704 M
HyperPP6	54 m	–	18	1 per month	1–2 weeks	Myotonia	None	(+) Stiffness	M 1360 V
HyperPP8	44 m	+	6	Daily	1/2 2 h	Myotonia atrophy	Severe	(+) Gen. paralysis	T 704 M
HyperPP9	22 m	+	10	2–3 per week	Hours	Myotonia	None	(+) Gen. paralysis	SCN4A
HyperPP10	28 m		8	1 per month	Days	None	Moderate	(+) Gen. paralysis	T 704 M
HyperPP11	52 m	+	2	1 per month	Hours	Myotonia atrophy	Moderate	(+) Gen. weakness	T 704 M
ThyPP 1	27 m	–	23	2 per year	1 day	Hyperthyroidism	None	(+) Severe weakness	
ThyPP 2	53 m		53	1 attack	Hours	Hyperthyroidism	None	(–)	

HypoPP = hypokalemic periodic paralysis; HyperPP = hyperkalemic periodic paralysis; ThyPP = thyrotoxic periodic paralysis.
*Measured [K⁺] levels during spontaneous attacks; f, m = gender indicating female or male.

Table 2. Clinical information and subsequent diagnosis for patients with unclear diagnosis prior to *in vitro* studies.

Patient	Age and sex	Family history	Onset age (yr)	Attacks		Other symptoms	Permanent weakness	Clinical provocation test	Resultant diagnosis	Mol. gen. confirmation
				Frequency	Duration					
A	63 m	(+)	18	4 per year	2–3 months	Myalgia	None	(–)	HypoPP5	–
B	31 m	(+)	9	Daily	2–3 months	None	Slight	(–)	HypoPP6	–
C	42 m	(–)	23	1 per month	1–2 days	Myalgia	None	(–)	HypoPP7	–
D	25 m	(–)	15	1 per year	2–3 days	None	Slight	(–)	HypoPP10	
E	48 m	(+)	1	1 per week	Hours	Myalgia, atrophy, neurogenic	Severe	(–)	HypoPP11	–
F	24 f	(+)	22	1 attack	Hours	None	None	(–)	HypoPP12	–
G	21 m	(+)	12	1 per year	1 week	None	None	(–)	HypoPP13	–
H	40 m	(–)	38	Rare	Days	None	None	(–)	HypoPP14	–
I	20 f	(–)	4	1 per month	Hours to days	None	None	(–)	HypoPP17	–
J	41 m	(+)	2	2 per year	Days	Myot; myalgia	None	(–)	HyperPP7	M 1592 V
K	54 f	( )	52	2 per week	10 min	Diarrhoea, depression	None	( )	NonPP1	:
L	45 m	(–)	45	1 per week	Minutes	None	None	(–)	NonPP2	–
M	42 m	(–)	42	1 attack	1 day	Alcoholism	None	(–)	AhypoPP1	–

*Gender indicating f for females and m for males. HyperPP = hyperkalemic periodic paralysis; HypoPP = Hypokalemic periodic paralysis; NonPP = non-dyskalemic periodic paralysis; AhypoPP = acquired hypokalemic periodic paralysis.

In some experiments a Cl⁻ free solution was used that was made by replacing NaCl and KCl with the respective methane sulfonate salts and $CaCl_2$ with Ca gluconate and by omitting the sugars in order to avoid hyperosmolarity. The solutions with higher or lower than normal potassium concentration were made by the addition or omission of the appropriate amounts of KCl to the standard solution. Li^+ containing solutions (1 or 2 mM) were made by the appropriate additions of LiCl. Drugs were added to the bathing solution in single doses to yield the desired final concentrations, such as tetrodotoxin (TTX; Roth, Karlsruhe, Germany); insulin (Merck, Darmstadt, Germany); dihydro-ouabaine (Sigma, St. Louis, MO, U.S.A.); adrenaline (Sigma); prednisolone (Merck); pinacidil (Biotrend, Köln, Germany); HOE 234 (Hoechst, Frankfurt a.M., Germany), EMD 52962 (Merck). The K^+ channel activator cromakalim (BRL 34915; Beecham Pharmaceuticals, Gronau, Germany) was prepared in dimethylsulfoxide (DMSO; Sigma). All solutions were maintained at 37°C if not indicated otherwise, and their pH was adjusted to 7.4 by gassing with 95% O_2 and 5% CO_2.

Force measurements

From each specimen, bundles of resealed muscle fibre segments [17] were mounted in several experimental chambers with one end fixed and the other end fastened to a force transducer (Grass FT03, Quincy, MA, U.S.A.). The signals from the transducers were amplified and recorded as described [18, 19]. Platinum plate electrodes on either side of the muscle bundles were used for direct ("field") stimulation with supramaximal pulses of 1 ms duration at a frequency of 0.1 Hz. Each bundle was stretched until the twitch amplitude was maximal. At this length, slight shortening or lengthening did not alter the peak force amplitude. With this arrangement several drugs could be tested in parallel.

RESULTS

Although all patients claimed to suffer every now and then from attacks of paralysis, such episodes were not elicited by clinical provocation in 13 out of our 30 patients (Table 2). In these patients a certain diagnosis of the type of periodic paralyses was only deducted from the *in vitro* measurements and compared to the results obtained with excised muscles from patients with clear diagnoses by altering the extracellular ion composition and/or applying various drugs.

Twitch forces in bundles from patients with clinically confirmed HypoPP

Exposure of bundles from HypoPP patients to a 1 mM K^+ solution usually induced a decrease in twitch force. In some cases this test produced little or no effect. Then, insulin (100 IU/l) or adrenaline (1 μM) was added to the bath which always lead to a dramatic loss of force [Fig. 1(A), (B), (D)].

The exposure of the bundles to a Cl free solution always induced a nearly complete paralysis. Addition of Li^+ (1 mM) or a K^+ channel activator (cromakalim, 100 μM; pinacidil, 100 μM; or EMD 52692, 1–10 μM; HOE 234, 10–50 μM) induced a significant recovery of the twitch force, often well beyond the original control amplitude produced in a 4.5 mM K^+ solution [Fig. 1(B), (D)].

In contrast, when the preparations were kept in standard solution, administration of adrenaline to HypoPP bundles enhanced the twitch [Fig. 1(C)], similarly as observed with normal and HyperPP bundles. The effects of altering the extracellular ion composition and/or the effects of various drugs are summarized in Table 3.

Table 3. A summary of the common changes in force in various solutions, with either altered ionic compositions or drugs added, observed for normal fibers and those from patients known to suffer from either HypoPP or HyperPP.

|	Normal	HypoPP	HyperPP
Low [K⁺]	None	Decrease	None
High [K⁺]	None	None	Decrease
Cl⁻ free	Transient	Paralysis	Paralysis
Adrenaline, normal [K⁺]	Increase	Increase	Increase
Low [K⁺] and insulin (100 μM)	None	Decrease	--
Low [K⁺] and adrenaline	None	Decrease	--
K⁺ channel activator	None	Increase	Increase
K⁺ channel activator and low or high [K]	High: decrease low: none	Low: increase	High: decrease
Lithium (1 mM)	None	Increase	Increase
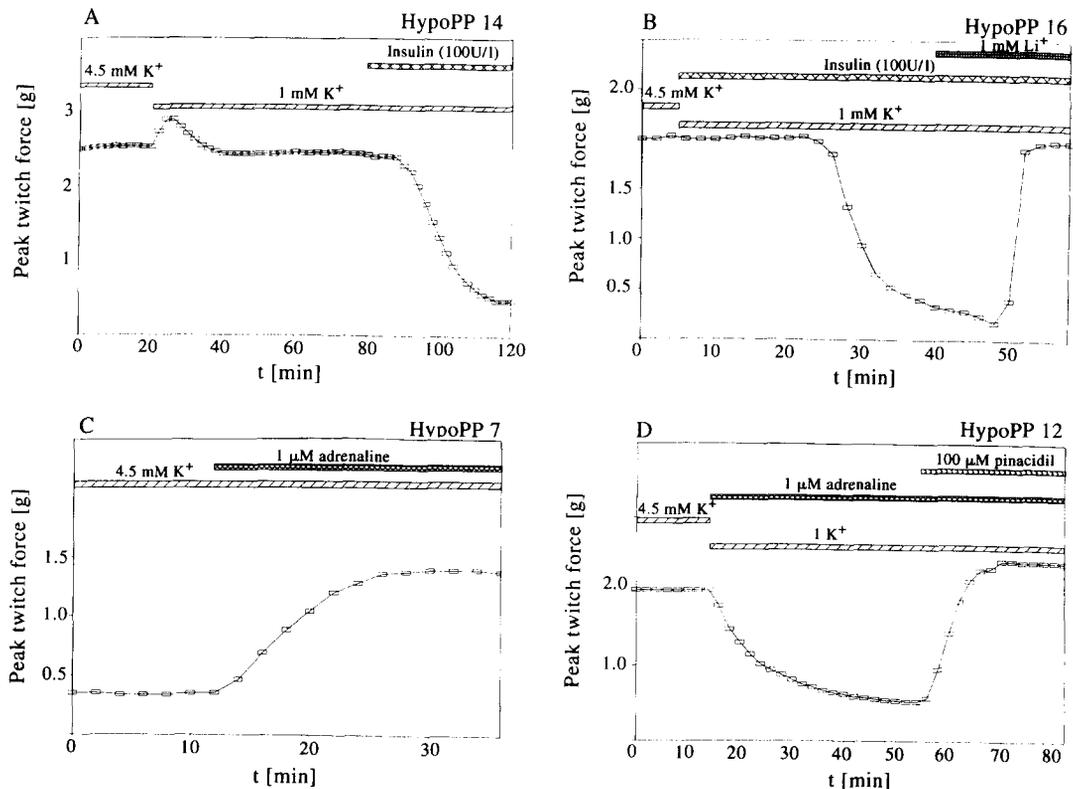
Fig. 1. Muscle bundles from patients with suspected hypokalemic periodic paralysis: changes in peak twitch force occurring upon alteration of the ionic composition of the bath or upon administration of drugs. Although force usually decreased upon admission of a low $[K^+]_e$ solution, this was not the case in (A) and (B); the addition of 100 U/l insulin induced nearly complete paralysis although sometimes with a delay (B). The administration of 1 μM adrenaline induced an increase in twitch force when $[K^+]_e$ was 4.5 mM (C), but when $[K^+]_e$ was 1 mM, it caused a decrease of force (D). The administration of Li^+ (1 mM) induced a recovery of the twitch even in the presence of low $[K^+]_e$ and insulin (B). Similar recovery was achieved by administration of 100 μM pinacidil (D).

Twitch forces in bundles from HyperPP patients with subsequently identified mutations

Exposing bundles from HyperPP patients to either an elevated $[K^+]_e$ (> 6.0 mM–9 mM) or to a Cl-free solution induced a pronounced weakness (> 50% decrease in twitch force; Fig. 2). The addition of 200 mg/l prednisolone to the high K^+ solutions resulted in an additional loss of force of the HyperPP bundles. Similarly as with HypoPP bundles, the addition of a K^+ channel activator (cromakalim, 100 μM; or pinacidil, 100 μM; or EMD, 1–10 μM; or HOE 234, 10–50 μM) to the HyperPP bundles induced a dramatic recovery of twitch force (Fig. 2 and Table 3).

Twitch behavior of normal muscle

In contrast to muscle bundles from PP patients, normal muscle responds to an exposure to a Cl-free solution with a transient (1–2 min) decrease of twitch force followed by an enhancement of force due to repetitive activity

(Fig. 3). The dramatic decrease in twitch force of bundles from HypoPP patients in the presence of a low $[K^+]_e$ or the decrease in force of HyperPP bundles in elevated $[K^+]_e$ never occurred in normal muscle. Some bundles of normal muscle developed a reversible slight decrease (30%) in twitch force when exposed to 9 mM K^+, whereas 7 mM K^+ had no effect. A comparison between the responses of HyperPP, HypoPP and normal muscle fibres to altered extracellular ionic composition and/or to a drug administration is given in Table 3.

Differential diagnosis

Prior to *in vitro* analyses, the diagnoses in several of these patients were ambiguous. For example, in 10 out of 15 of the HypoPP patients the attempt to provoke a paralytic attack did not lead to weakness or other typical signs (in one patient the provocation test was unsuccessfully repeated on four different occasions). It should be noted, however, that the
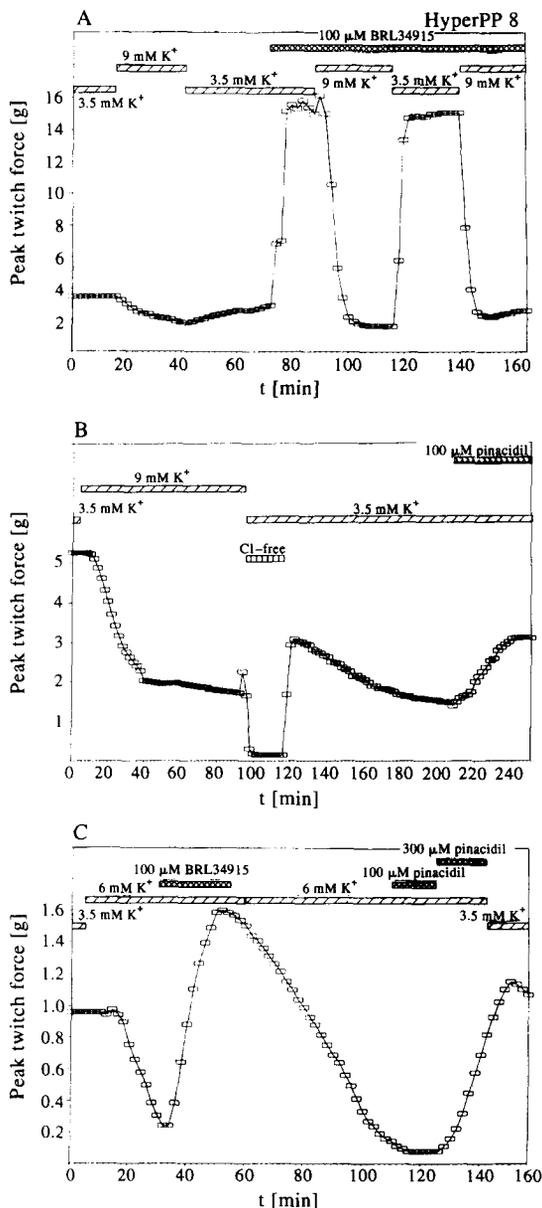
Fig. 2. Muscle bundles from patients with known hyperkalemic periodic paralysis: changes in peak twitch force occurring upon alteration of the ionic composition of the bath or upon administration of drugs. Increasing [K$^+$]$_e$ from 3.5 to 9 mM causes further decrease of the low twitch amplitude (A). Twitch force is restored in 3.5 mM [K$^-$]$_e$, but not in 9 mM [K$^+$]$_e$, when 100 μM BRL 34915 (cromakalim, a K$^+$ channel activator) is applied (A). Removal of [Cl$^-$]$_e$ produces near complete paralysis lasting until Cl$^-$ is readmitted (B). Addition of 100 μM pinacidil stabilizes twitch force (B) or is ineffective (C). At 300 μM, pinacidil restores force even in the presence of 6 mM [K$^+$]$_e$ (C).

Fig. 3. Response of a repetitively stimulated control muscle bundle to a Cl$^-$-free solution. Upon washout of Cl$^-$, the twitch force decreases within 5 min and then slowly recovers. Myotonic activity (i.e. slowed relaxation) then becomes evident (black band above base line). Myotonia persists until extracellular Cl$^-$ is readmitted.

ability to provoke a response clinically was not related to the severity of attacks of the patients: patients HypoPP6 and HypoPP10 had negative clinical tests, but often experienced severe attacks (lasting days). In addition, in two of
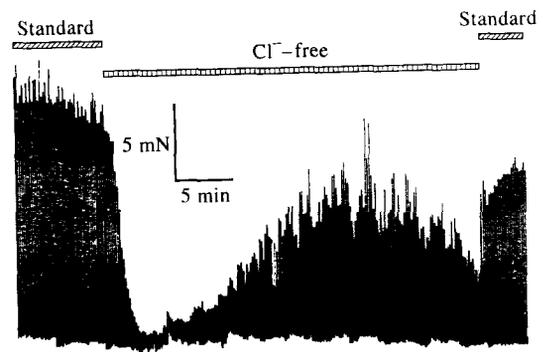
our patients who claimed to have suffered from paralytic attacks, such episodes could not be clinically provoked, and all *in vitro* twitch tests gave normal results. As a consequence, we considered the origin of their weakness as nondyskalemic (NonPP, Table 1).

In three other patients without apparent genetic basis the conditions were considered as acquired since permanent hypokalemia (AhypoPP1) or thyrotoxicosis was present (ThyPP1,2). Interestingly the muscles of these patients behaved *in vitro* similarly as the muscles from genetically affected HypoPP patients.

For example, muscle bundles obtained from patient AhypoPP1 became weak (a 30% decrease in peak twitch amplitude) when exposed to a 1 mM K$^+$ solution and were paralysed when insulin was added.

As with genetically determined PP, muscle from patients with acquired PP revealed a dramatic recovery of twitch force in the presence of K$^+$ channel activators. But in contrast to genetically determined HypoPP, the muscle from the ThyPP patients developed a slight increase in twitch force when adrenaline was added to the low [K$^+$] solution (Table 3.).

DISCUSSION

Our results show that *in vitro* investigation of skeletal muscles can clarify the situation of patients who claim to suffer from attacks of weakness but whose diagnosis cannot be established clinically. It should be noted that many of our methods used for testing the twitch force

(i.e. modifying the ionic composition of the bath solution or *in vitro* administration of drugs) have been previously applied [17, 27, 29]. However, so far they have not been described as tools for discriminatory diagnostic tests. Not all the solution changes or drug additions that we have used here need always be performed for *in vitro* clarification of the diagnosis. For example, for several individuals with ambiguous diagnosis of HypoPP, lowering of the $[K^+]_e$ induced a complete paralysis and this can be considered sufficient for a confirmation of the diagnosis. Only when there is little or no reduction of force, is the further administration of either insulin or adrenaline indicated. The test results were shown to be conclusive independently of the severity of a patient's clinical signs, symptoms or features.

For each form of PP, we found that the expression of signs and symptoms varied considerably (see Tables 1 and 2). For example, patient HypoPP11 suffered from severe progressive weakness and atrophy, whereas patient HypoPP12 had only once experienced an attack of weakness for several hours and showed no other symptoms. This well-known *in vivo* variability may be indicative of differing stages of progression or different subtypes [20, 21]. The important finding in our context is, that quite independently from this expression, the muscles from all these patients revealed a significant decrease in twitch force in a low $[K^+]$ solution.

PPs are sometimes associated with a cardiomyopathy [22]. None of our 14 HypoPP patients showed signs of such a complication, as previously reported [23]. However, in one of the HypoPP patients a cardiac arrhythmia was noted during a severe paralytic attack in which serum K^+ dropped to 1.7 mM (dizziness was also noted). Fortunately, the majority of our HypoPP patients did not require and/or want any medication. In any case we advised our patients to reduce their intake of carbohydrates. Acetazolamide, in controlled dosages, was effective in decreasing the severity of attacks of weakness in three out of five cases, and diclofenamide was given to three others for the treatment of their permanent weakness.

Studies of venous and arterial blood revealed that hypokalemia can be generated in affected patients by an insulin-stimulated inflow of K^+ from the outside (extracellular space) to the inside of the muscle cells [24, 25]. Increased

insulin binding was reported for the musculature of a HypoPP patient, however, it was not clear whether the number or the affinity of the insulin receptors was increased [26]. We observed that *in vitro* the addition of insulin to a low $[K^+]$ solution further decreased muscle twitch force because insulin may lower the K^+ outward conductance. *In vivo*, insulin lowers $[K^+]_e$ via stimulation of the Na^+/K^+ pump. Both effects may lead to a depolarization of the muscle cells as discussed in ref. [27]. Details of the insulin action, however, remain unclear. The administration of ouabaine had little or no effect on HypoPP muscle which suggests that the Na^+/K^+ –ATPase pump may not be primarily involved in this disease.

The decrease of the *in vitro* twitch force of muscle bundles from familial HypoPP patients upon addition of adrenaline to a low $[K^+]$ solution is consistent with the report that adrenaline can specifically provoke weakness in familial HypoPP [28]. This finding is contrasted by our observation of an increase in force in muscle bundles from a ThyPP patient. The augmentation of twitch force in HypoPP, HyperPP, ThyPP and normal muscle that was consistently seen when adrenaline was added to the standard solution (3.5 mM K^+) may be best explained by the inability of the Na^+/K^+– ATPase to reduce $[K^+]_e$ substantially in the relatively large experimental chamber.

As previously reported [29], K^+ channel activators were effective in increasing *in vitro* twitch amplitudes of muscle from PP patients. A new substance, HOE 234 was now found equally effective, though in lower concentration than cromakalim. For a similar *in vitro* benefit (potency), the effective concentrations of these activators are: 5 μM EMD 52962, 50 μM HOE 234 and 100 μM cromakalim. Unfortunately, the use of these agents for the treatment of a paralytic attack in HypoPP may be limited in view of the clinical side effects. Nevertheless, these agents did not only dramatically augment the twitch in standard solution, they also prevented the decrease in force in HypoPP bundles in a low K^+ solution. As predicted by the Nernst equation, they were not able to prevent weakness or to restore force when extracellular K^+ was as high as 9 mM.

The finding that the *in vitro* administration of prednisolone to HyperPP bundles in high K^+ solutions further reduced twitch force is consistent with at least one report in which this agent

is considered provocative [30]. Also in agreement with this report, one patient (HyperPP12) reported that fludrocortisone was effective in treating his symptoms, in particular his permanent weakness.

As to the usefulness of Li⁺ for treatment in PP, the reports are conflicting. Ottoson and Persson, reported the lack of therapeutic benefit for treating HypoPP patients, whereas Confavreux and co-workers reported a significant reduction of paralytic attacks, but no influence on permanent weakness [31, 32]. The recovery of twitch force in an *in vitro* experiment in the presence of Li⁺ is consistent with the latter report, but requires further clarification. In most other types of excitable tissues, the administration of extracellular Li⁻ induces depolarization [33]. Perhaps, the way by which Li⁺ can augment twitch force in HypoPP is that it mimics the properties of K⁺. An additional benefit of Li⁺ treatment *in vivo*, which would not be relevant *in vitro*, is an ultimate reduction in the excretion of K⁺, hence minimizing changes in the plasma [K⁺].

In conclusion, the described *in vitro* investigation of muscle bundles from patients suffering from unexplained attacks of weakness can provide insight into the underlying cause and make a differential diagnosis possible.

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