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Review

An expanding view for the molecular basis of familial periodic paralysis

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Abstract

The periodic paralyses are rare disorders of skeletal muscle characterized by episodic attacks of weakness due to intermittent failure of electrical excitability. Familial forms of periodic paralysis are all caused by mutations in genes coding for voltage-gated ion channels. New discoveries in the past 2 years have broadened our views on the diversity of phenotypes produced by mutations of a single channel gene and have led to the identification of potassium channel mutations, in addition to those previously found in sodium and calcium channels. This review focuses on the clinical features, molecular genetic defects, and pathophysiologic mechanisms that underlie familial periodic paralysis.

Keywords: Andersen's syndrome; Ion channels; Myotonia; Paramyotonia; Skeletal muscle

1. Periodic paralysis is a disorder of skeletal muscle excitability

The fidelity and rapidity of skeletal muscle contraction in response to release of acetylcholine from axon terminals of motor neurons is critically dependent on the electrical excitability of the muscle fiber. The depolarizing postsynaptic potential at the motor endplate triggers an action potential which propagates longitudinally along the surface of the fiber and radially inward along the transverse tubules where membrane depolarization induces a conformational change in L-type Ca channels that triggers Ca^{2+} release from the sarcoplasmic reticulum (SR).

The episodic attacks of flaccid weakness that are the clinical hallmark of familial periodic paralysis are due to a transient loss of muscle fiber electrical excitability (reviewed in [1–3]). During an attack of weakness, affected fibers are depolarized at −50 to −60 mV from a normal resting potential of −90 mV [4]. Depolarization inactivates Na channels and thereby prevents the generation or propagation of action potentials. The end result is flaccid weakness, similar to the state of muscle produced by exogenous depolarizing neuromuscular blockers such as succinylcholine. Familial periodic paralysis is inherited as a dominant trait, and the intermittent failure to maintain the skeletal muscle resting potential is due to mutations in genes coding for voltage-gated ion channels. Inherited defects in voltage-gated sodium (Na) channels, calcium (Ca) channels, or potassium (K) channels may result in periodic paralysis. Regardless of which type of channel is defective, the final common pathway is depolarization-induced loss of muscle excitability. Sporadic cases of periodic paralysis may be due to de novo mutations of ion channel genes or to secondary causes such as thyrotoxicosis or Ba^{2+} poisoning. Clinical signs of thyrotoxicosis are not always present in patients with thyrotoxic periodic paralysis, and therefore thyroid function studies must be performed to distinguish thyrotoxic from sporadic familial periodic paralysis.

Several variants of familial periodic paralysis have been delineated on the basis of clinical features: serum potassium during an attack, stimuli that precipitate attacks, or maneuvers that foreshorten attacks for example. The expectation was that these variants might be due to involvement of different classes of ion channels. The reality of the situation has proven to be more complicated. Mutation-induced defects in the same channel may give rise to diverse phenotypes (allelic disorders). For example, gain-of-function defects in voltage-gated Na channels may cause myotonia (a state of abnormally enhanced muscle excitability), periodic paralysis, or both. Conversely, mutations in different channel genes may produce a common phenotype. Mutations in either Na or L-type Ca channel genes are both causes of hypokalemic periodic paralysis (HypoPP) with similar, if not identical, clinical features.

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2. Clinically delineated forms of familial periodic paralysis

The familial periodic paralyses are rare inherited disorders of skeletal muscle that present with recurrent episodes of muscle weakness [5]. The weakness is often generalized, but the arms or legs may be preferentially affected in a particular attack. Respiratory and bulbar muscles (extraocular movements and deglutition) are usually spared. The inheritance is autosomal dominant with the onset of symptoms in the first or second decade. Several variants of familial periodic paralysis have been delineated clinically. The different variants are distinguished by precipitating factors, serum potassium levels during an attack, and additional signs such as myotonia or cardiac arrhythmia (Table 1). These distinctions have important practical implications, because the recommendations to patients on how to minimize the frequency, severity, and duration of attacks depends of the form of familial periodic paralysis.

2.1. Hypokalemic periodic paralysis

HypoPP is the most prevalent form of familial periodic paralysis, but it is still a rare disorder (prevalence estimated to be ~1 per 100,000). Attacks usually begin around puberty, but in severe cases weakness may occur in childhood. An attack of weakness may be provoked by rest after strenuous exercise, carbohydrate-rich meals, ethanol use, or stress. Inheritance is autosomal dominant, but in some families affected women only develop late onset proximal myopathy and never have attacks of weakness [6]. For patients with episodic weakness, the attack frequency may range from only a few in a lifetime to almost daily attacks.

The most specific clinical features of HypoPP are low serum [K^+] with onset of an attack (often <3.0 mEq/L) and clinical improvement with oral K^+ supplementation. Both features are variable, however, and their absence does not exclude a diagnosis of HypoPP. The duration of weakness is highly variable. However, episodes of weakness in HypoPP are often more protracted (several hours to days) than with other forms of periodic paralysis. Permanent proximal myopathy occurs frequently in HypoPP. Symptoms, such as difficulty climbing stairs or rising from a chair, typically begin in the fourth or fifth decade and the rate of progression is slow. The serum CK is usually normal or slightly high and muscle biopsy shows vacuoles, increased variation in fiber size and central nuclei. Myotonia does not occur in HypoPP, either symptomatically or electromyographically. The baseline EKG is normal in HypoPP, but secondary changes may occur as a result of hypokalemia during an attack of weakness (lowered T waves, prolongation of QT interval). Hyperthyroidism may also cause episodic weakness with hypokalemia, especially in young Asian males, and thyroid function must be assayed to exclude thyrotoxic periodic paralysis.

2.2. Hyperkalemic periodic paralysis (HyperPP)

The clinical hallmarks of HyperPP are episodic weakness in association with hyperkalemia (>5 mEq/L) and dominant inheritance. Serum potassium is not always high during a spontaneous attack, and a more consistent finding is provocation of weakness by K^+ administration. Weakness may also be triggered by rest after exercise, stress, fasting, or muscle cooling. The first attack often occurs in infancy or early childhood. Episodes last from minutes to hours, and are generally shorter than attacks in HypoPP. Myotonic stiffness of the hands and face may occur with HyperPP. The interictal electromyogram (EMG) often shows runs of myotonic discharges. At the beginning of an attack of weakness, muscle electrical irritability and myotonia may increase. During this interval patients may report a sense of tingling or muscle tension. As the attack progresses to weakness, muscle excitability is lost. The frequency of episodic attacks usually diminishes with age, and permanent proximal weakness with vacuolar myopathy may develop in HyperPP.

HyperPP is not associated with primary defects in other electrically excitable tissues such as nerve or cardiac muscle. Hyperkalemia during an attack of weakness may produce secondary EKG changes (peaked T waves, U waves, atrial or ventricular ectopic beats), but there is no baseline rhythm disturbance or cardiac conduction defect in HyperPP.

Table 1
Clinical variants of familial disorders with periodic paralysis

	Paramyotonia congenita	Hyperkalemic periodic paralysis	Hypokalemic periodic paralysis	Andersen's syndrome
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Age at onset	First decade	First decade	Second decade	Second decade
Serum [K^+] (ictal)	Usually normal, may be high	High or normal	Usually low	Variable
Triggers	Cold, exercise	K^+ ingestion, rest after exercise	Carbohydrate ingestion, insulin, rest after exercise, high Na^+ diet	Exercise
Episodic weakness	Mild to moderate	Moderate to severe, improvement within hours	Often severe and protracted (many hours to days)	Usually infrequent
Myopathy	Very rare	Occasionally, proximal	Frequent, proximal, late onset	Occasionally
Myotonia	Paramyotonia, cold-aggravated	± Myotonia	None	None
Dysmorphic features	None	None	None	Face, hands, stature
Cardiac arrhythmia	None	None	None	LQT, ventricular arrhythmias

2.3. Paramyotonia congenita (PMC)

PMC shares considerable clinical overlap with HyperPP, and the two disorders are allelic (i.e. both arise from mutations in the same gene). Patients with PMC often have episodic attacks of weakness with a duration, severity, and serum [K^+] change comparable to those observed in patients with HyperPP. Unlike HyperPP, however, the predominant symptom in PMC is paramyotonia–myotonic stiffness that paradoxically worsens with repeated muscle contraction. Paramyotonia is usually most pronounced in muscles of the hands and face. Cold weather or provocative testing by limb immersion in ice water markedly aggravates paramyotonia. In contrast, myotonia is greatest with the first few voluntary contractions after rest in myotonia congenita (warm-up phenomenon) and is relatively insensitive to cold.

2.4. Andersen's syndrome (AS)

AS is the most recently delineated variant of familial periodic paralysis and is unique among the periodic paralyses because tissues other than skeletal muscle are affected as well. The first clues to the existence of this subgroup were reports of familial periodic paralysis with ventricular arrhythmia, independent of the serum [K^+]. In 1971, Andersen described a family with the triad of periodic paralysis, ventricular ectopy, and developmental abnormalities [7]. Subsequently, Tawil's group identified 15 similar cases from eight different kindreds, which formed the basis for their definition of AS as the triad of potassium-sensitive periodic paralysis, ventricular arrhythmia and dysmorphic features [8,9]. Inheritance is autosomal dominant, but penetrance is highly variable. The clinical recognition of AS usually occurs when the proband has all three features. Other affected family members (proven genetically) often have only two or even one of the clinical features of AS.

The skeletal muscle dysfunction in AS is similar to other forms of periodic paralysis. Attacks of weakness begin in the first or second decade. Serum [K^+] during a spontaneous attack is often low, but may be normal and in some cases has even been high. The response to provocative hypo- or hyperkalemic challenges has not been possible to systematically assess, because the coexisting cardiac defects render such testing too risky. Mild permanent weakness is another common feature of AS that was observed in 50% of patients. Myotonia is not present symptomatically or by EMG. The CK is usually normal, and the muscle biopsy typically shows mild chronic myopathy with tubular aggregates.

Cardiac involvement in AS spans a wide spectrum of ventricular arrhythmias from asymptomatic long QT (LQT) to life-threatening ventricular tachycardia. In a series of 15 patients, the QT interval was prolonged in 12 [9]. Other cardiac defects observed in AS patients include ventricular ectopy, bi-directional ventricular tachycardia, and recurrent *torsades de pointe*. In patients with cardiac arrest, an implantable defibrillator may be required.

A constellation of distinctive dysmorphic features has been observed in AS. The most commonly involved structure is the face, which in AS may show low-set ears, broad nose, hypertelorism (wide-set eyes), and micrognathia (small mandible). Deformity of the digits also occurs with clinodactyly of the fingers or syndactyly of the toes. Less commonly occurring physical features include short stature, scoliosis, and high-arched palate.

3. Molecular genetic defects in periodic paralysis: mutations of voltage-gated ion channels

The molecular defects that give rise to familial periodic paralysis are all mutations in the coding regions of ion channel genes (Fig. 1). Most are missense mutations, with the one exception being small in-frame deletions in a potassium channel that occurs in AS. There are no known examples of more severe mutations that would produce truncated or radically altered proteins (e.g. frame-shift mutations, premature stop codons, or splicing defects) or mutations in the non-coding regulatory regions of the genes.

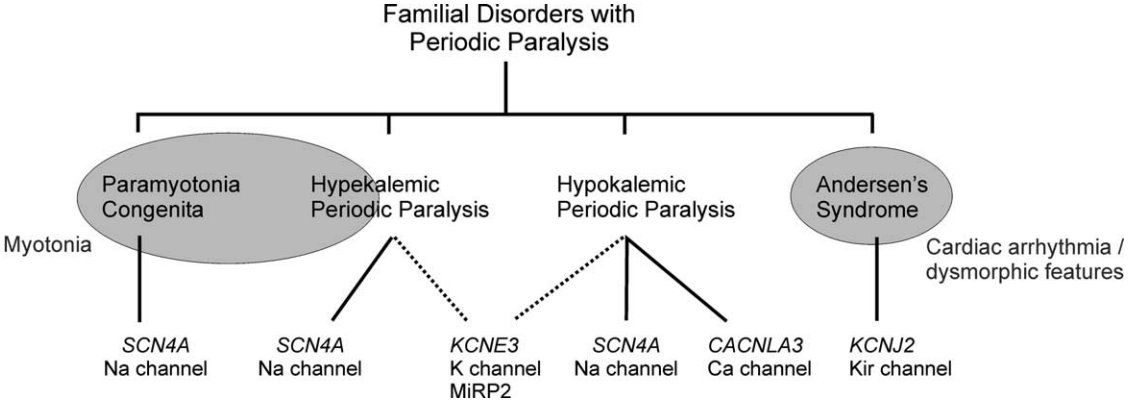

Fig. 1. Tree diagram showing the relation between clinical features (shaded circles) and gene defects for familial disorders with periodic paralysis. Gene definitions: *SCN4A*-α subunit of the adult skeletal muscle isoform of the Na channel, *KCNE3*-K channel subunit MiPR2 (MinK-related peptide 2), *CACNLA3*-α1S subunit of the L-type Ca channel in skeletal muscle, *KCNJ2*-inward-rectifying K channel Kir2.1. The association of *KCNE3* mutations with HyperPP or HypoPP is indicated by dashed lines because for each instance only a single family has been identified.

Historically, the first mutations were identified in families with HyperPP. Pioneering electrophysiological studies on biopsied muscle fibers by Lehmann-Horn and coworkers demonstrated an aberrant tetrodotoxin-sensitive inward current in HyperPP fibers that was never observed in normal muscle [10]. This observation implicated a defect in the voltage-gated Na channel. The Na channel in skeletal muscle is a heterodimer of the pore-forming α subunit and an accessory β_1 subunit. Using a candidate gene approach, both HyperPP and PMC were found to be tightly linked to the adult isoform of the skeletal muscle Na channel α subunit gene (*SCN4A*) on chromosome 17q23 [11,12]. The *SCN4A* gene comprises 24 exons spanning 35 kb and codes for a protein of 1836 amino acids. Mutational analyses of genomic DNA have identified nine missense mutations associated with HyperPP and seven in PMC with at least one episode of weakness, whose locations are shown on the membrane-folding diagram for the Na channel in Fig. 2. Some mutations occur more frequently than others. T704M and M1592V are the two most commonly identified mutations in HyperPP, and together account for nearly two-thirds of positively genotyped kindreds. For PMC, T1313M and R1448C are the most prevalent missense mutations. Haplotype analysis has excluded a common founder for these more prevalent mutations.

For some HyperPP or PMC families a mutation in *SCN4A* has not been found. This discrepancy may be due to technical limitations of the genetic screen, clinical misdiagnosis, or phenocopy produced by mutations in other channel genes. Based on the hypothesis that a K channel defect might also produce periodic paralysis, a panel of 100 patients without mutations in *SCN4A* (or a Ca channel gene *CACNLA3*) was screened for defects in *KCNE3*, the gene coding for a K channel accessory subunit called MiRP2 (MinK-related peptide 2) [13]. Two families were found to have a missense mutation, R83H. The phenotype for one family was typical for HypoPP whereas the other was suggestive of HyperPP (attacks alleviated by carbohydrate-rich meals, but serum $[K^+]$ was normal during attacks and a K-challenge was not performed). Aside from this one possible exception, the only molecular defects identified in families with HyperPP or PMC are missense mutations in *SCN4A*.

Mutations associated with HypoPP were identified by genetic linkage analysis and positional cloning. In the absence of electrophysiological data to implicate a particular channel defect, linkage to chromosome 1q31-32 was established using a genome-wide screen of dinucleotide repeat markers [14]. A Ca channel gene, *CACNLA3* (also termed *CACNLAS*) coding for the α1S subunit of the skeletal muscle L-type Ca channel had previously been mapped to this region. Analysis of genomic DNA has identified three missense mutations of *CACNLA3* in HypoPP families [15,16]. R528H and R1239H are equally common, while

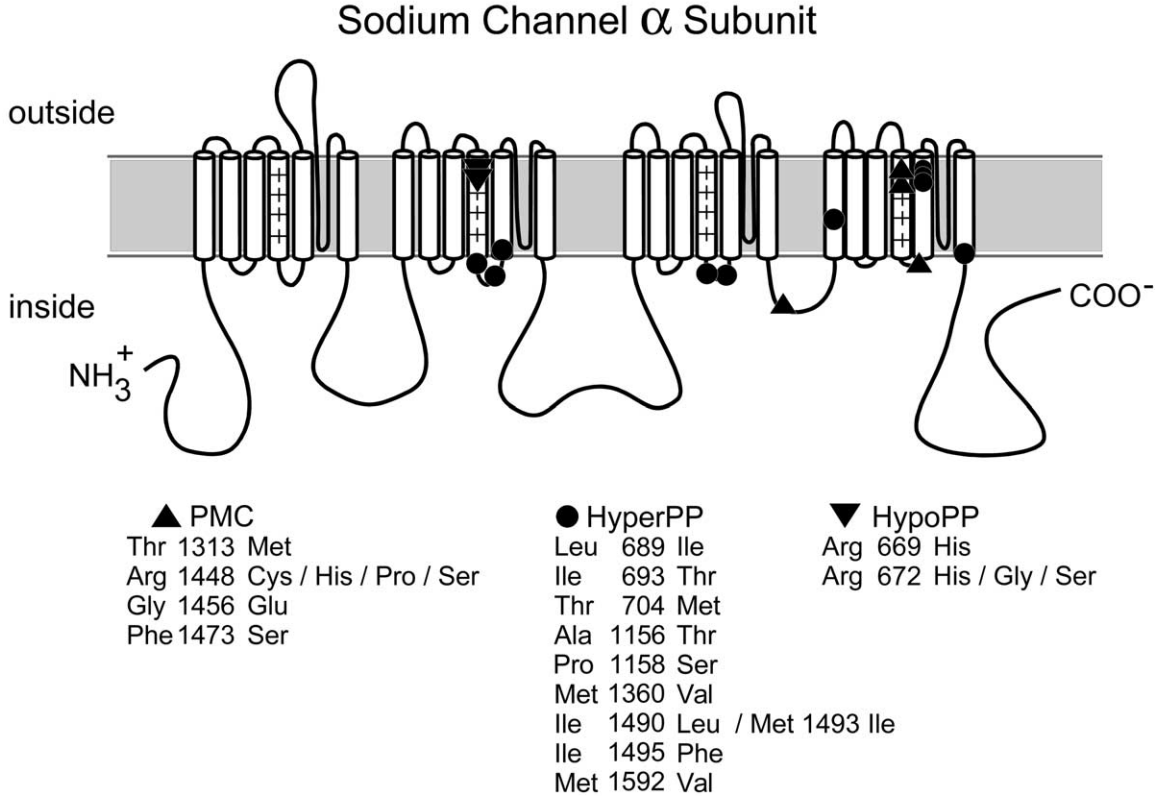

Fig. 2. Membrane-folding diagram for the α subunit of the skeletal muscle Na channel showing the locations of missense mutations found in HyperPP, PMC, and HypoPP. The PMC mutations are limited to a subset for which at least one episode of periodic paralysis has been reported.

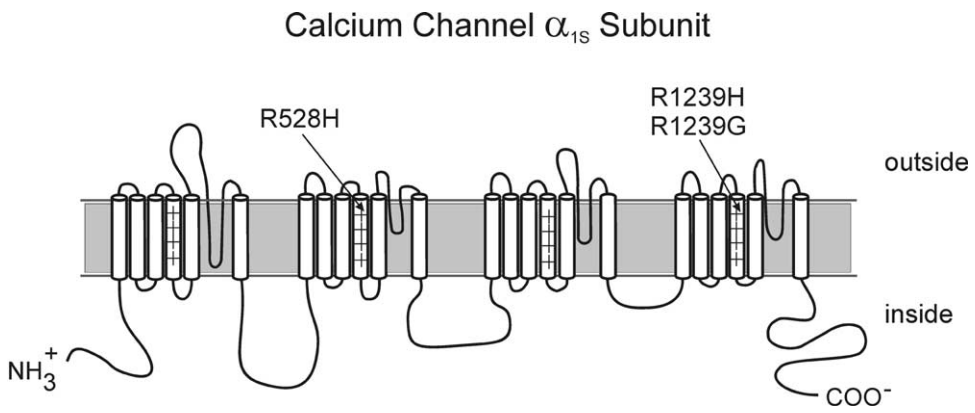

Fig. 3. Membrane-folding diagram for the α1S subunit of the skeletal muscle L-type Ca channel showing the locations of missense mutations found in HypoPP.

R1239G is a much rarer mutation. Interestingly, all three mutations occur at arginine residues, which are positively charged and form part of the voltage-sensing apparatus of the channel (Fig. 3). Missense mutations in *CACNLA3* are also a rare cause of susceptibility to malignant hyperthermia [1]. The L-type Ca channel is a heteropentamer comprising the pore-forming α1S subunit and four accessory subunits: α_2, δ, β, and γ. Mutations in these accessory subunit genes have not been found in human neuromuscular disorders.

The molecular defect in HypoPP is not always a mutation of *CACNLA3*. In about 30% of HypoPP families none of the three missense mutations in *CACNLA3* have been found [6]. Some kindreds are large enough to demonstrate that the HypoPP locus is not linked to chromosome 1q31-32. Screening these patients (either not linked to 1q31-32 or linkage indeterminate) for mutations in the Na channel gene, *SCN4A*, revealed four additional mutations at two residues: R669H, and R672 mutated to H, G, or S (Fig. 2) [6,17,18]. Lehmann-Horn and coworkers have designated this group as HypoPP-2, to distinguish it from the Ca channel mutations, HypoPP-1. Curiously, the R669H mutation in the voltage sensor of the Na channel is in a position exactly homologous to the HypoPP-associated R528H mutation in the L-type Ca channel. Sodium channel mutations should be regarded as an infrequent cause of HypoPP. In a recent study of 58 independent HypoPP index cases, 40 were linked to *CACNLA3* and only five were linked to *SCN4A* [6]. One large family with the R672G mutation in *SCN4A* was notable in that attacks of weakness were followed by myalgias and acetazolamide increased the severity and frequency of attacks. Finally, a missense mutation in a K channel accessory subunit, MiRP2 (*KCNE3*), was found in one family with symptoms consistent with a diagnosis of HypoPP [13].

The disease gene in AS was also identified by a combination of genetic linkage and positional cloning [19]. Linkage analysis of one large three-generation family with 15 affected individuals mapped the AS locus to chromosome 17q23. Three ion channel genes had previously been mapped to this locus: a sodium channel *SCN4A*, a calcium channel *CACNG1*, and a potassium channel *KCNJ2*. Because *SCN4A* had previously been excluded as a cause for AS and *CACNG1* is not expressed in heart, genetic screening was focused on *KCNJ2*. The *KCNJ2* gene is intronless and codes for a 427 amino acid protein that is an inward rectifying K channel, Kir2.1, expressed predominantly in heart, brain, and skeletal muscle. Nine different mutations of Kir2.1 were identified in a screen of 16 AS families. Seven of the mutations were missense and two were in-frame deletions, as shown in Fig. 4. No consistent phenotypic differences were discerned between families with missense mutations and those with small in-frame deletions. Although Kir2.1 is expressed in brain, there are no central nervous system manifestations in AS. For three of the 16 probands, no mutation was found in *KCNJ2*, suggesting genetic heterogeneity in AS.

4. Functional defects of mutant channels and the pathogenesis of periodic paralysis

Most functional studies of mutant channels have been performed in heterologous expressions systems that are

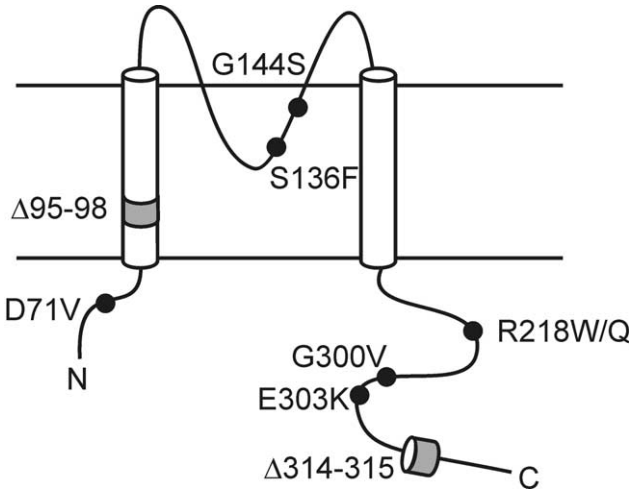

Fig. 4. Membrane-spanning topology of the Kir2.1 inward rectifying potassium channel showing the location of mutations identified in Andersen's syndrome.

chosen for their high efficiency of expressing ion channels at the surface membrane, rather than for providing a cellular environment similar to skeletal muscle. Although potentially important modifying factors or posttranslational processing and trafficking systems are different from those in skeletal muscle, these artificial systems provide the advantage of providing a uniform population of mutant channels at high density. The limited availability and viability of human muscle biopsy specimens combined with the lack of laboratory animal models for periodic paralysis has hampered efforts to record the behavior of mutant channels in fully differentiated muscle fibers. A few heroic studies have been performed on acutely dissociated biopsy specimens (e.g. [10]) or on primary cultures of myoblasts (e.g. [20]). In most respects, the behavior of mutant or wild-type channels observed in the few studies using skeletal muscle is indistinguishable from that recorded in artificial expression systems. This consistency bolsters our confidence in using the heterologous expression data to predict the functional defects in skeletal muscle and therefore the consequences for cellular excitability.

The final common pathway for the flaccid weakness in all forms of familial periodic paralysis is an inability to propagate action potentials from the motor endplate. This defect is due to sustained membrane depolarization, which inactivates a sufficient fraction of sodium channels to render the fiber inexcitable. Many of the biophysical abnormalities observed in heterologously expressed mutant channels are predicted to produce aberrant membrane depolarization. In general, the disease-associated mutations alter the gating properties of the channel (opening and closing in response to voltage), the expression level, or in the case of K channel mutants may produce non-functional subunits. The sodium channel mutations usually cause gain-of-function defects (impaired inactivation or enhanced activation) and the resulting inward current depolarizes the fiber. Conversely, loss-of-function defects are produced by the potassium channel mutations, which would reduce the resting K conductance and thereby depolarize the resting potential. The calcium channel mutations result in lower current density and slower activation compared to wild-type channels, but how this change leads to an instability of the resting potential is not readily apparent.

4.1. Hyperkalemic periodic paralysis/paramyotonia congenita

HyperPP and PMC are allelic disorders with overlapping clinical features, so it is not surprising that the defects of sodium channel behavior are similar for both sets of missense mutations. Sodium channels are closed at the resting potential and open rapidly, in a fraction of a millisecond, in response to membrane depolarization. Depolarized channels quickly inactivate to a non-conducting state within a millisecond or two. These fast-inactivated channels will not conduct current unless they are first reprimed by membrane hyperpolarization, which promotes recovery from fast inactivation back to the closed state over several milliseconds. The rapid development of fast inactivation limits the duration of the action potential, and the more sluggish recovery from fast inactivation at the resting potential gives rise to the refractory period.

The most commonly observed functional change for HyperPP/PMC mutants is an impairment of fast inactivation [21]. The impairment may affect different aspects of fast inactivation, and these differences are believed to be important determinants for the relative tendency for paralysis versus myotonia. The PMC mutations dramatically slow the development of fast inactivation (typically five-fold slower at 0 mV), but fast inactivation is eventually complete [22,23]. Conversely, recovery of PMC mutants from fast inactivation is often two or three times faster than wild-type channels at -100 mV. Computer model simulations of muscle fiber excitability show that these alterations in the rate of fast inactivation promote repetitive firing [24]. The slower onset of fast inactivation slightly broadens the action potential duration, which dramatically increases the egress of myoplasmic K^+ into the transverse tubular space per discharge. Potassium trapping in the transverse tubules (an extracellular space) depolarizes the fiber. The enhanced rate of recovery from fast inactivation for PMC mutants reduces the refractory period. These two effects synergistically promote the autonomous repetitive firing of action potentials, as observed by EMG in the myotonic run.

Mutations associated with HyperPP disrupt the completeness of fast inactivation or alter its voltage dependence. Fast inactivation of wild-type channels is nearly 100% complete at depolarized potentials. At the end of a 10-ms depolarization to 0 mV, for example, only about 0.1% or fewer wild-type sodium channels will be open. For HyperPP mutants, however, 1.5–5% of channels may remain open under the same conditions [20]. The persistent Na current conducted by this small fraction of non-inactivating mutant channels is sufficient to chronically depolarize the membrane potential by tens of millivolts. From this aberrantly depolarized condition, the majority of sodium channels will be inactivated (both wild-type and most of the HyperPP mutant channels). Consequently the fiber is inexcitable, unable to generate action potentials, even in response to direct galvanic electrical stimulation. The dominant expression of depolarization-induced weakness in HyperPP is due to a gain-of-function defect (too much inward Na current), which suppress the activity of normal sodium channels via membrane depolarization. Another defect of fast inactivation in HyperPP (and PMC) is a shift in the voltage dependence toward more depolarized potentials by 5–10 mV or more. This shift increases the so-called window current, a persistent Na current detected over a narrow range of modestly depolarized voltages (-50 to -40 mV) because the depolarization is not large enough to completely fast inactivate channels and yet is large enough to cause a measurable amount of Na channel opening. Over this

voltage range, the end result is comparable to the previously described defect produced by incompleteness of fast inactivation. The window current is precisely at the range of membrane potentials to which HyperPP fibers depolarize during an attack of weakness.

The segregation of these different forms of impaired fast inactivation to HyperPP versus PMC mutant sodium channels is not sharply divided. Fast inactivation defects of all types occur to varying degrees for each of the mutations associated with HyperPP and PMC. On the other hand, the distinctions listed in the previous paragraph are consistently observed among many different laboratories and experimental preparations. The depolarization during attacks of paralysis is an aberrant steady-state condition of the fiber, so it makes sense that the subset of mutations for which paralysis is the predominant symptom should have altered steady-state behavior (incomplete fast inactivation and shifts in voltage dependence) as the predominant channel defects. Conversely, myotonia is due to a dynamic instability of muscle excitability. PMC mutants have pronounced effects on the kinetics of fast inactivation, but not on the steady-state properties. There is also a quantitative difference. In general, channel defects associated phenotypes in which myotonia predominates are milder than those associated with paralytic phenotypes.

Two additional gain-of-function defects in channel behavior are found in mutations that cause HyperPP. A shift in the voltage dependence of activation toward more hyperpolarized potentials occurs in some HyperPP channels, and is particularly prominent for the most prevalent HyperPP mutation, T704M [25], and for I693T [26]. Just as with the depolarized shift of fast inactivation, the shift of activation will increase the persistent Na current in the window of overlap between activation and fast inactivation. There is an important difference, however. The hyperpolarized shift of activation brings the range of persistent window currents to more negative potentials, closer to the normal resting potential and farther from the voltage range where potassium channels are activated. These differences strongly increase the propensity for aberrant depolarization of the fiber. Computer simulations show that a hyperpolarized shift of activation by as little as 5 mV will produce an aberrant resting potential that is depolarized about 20 mV from its normal value. Conversely, a relatively large depolarized shift of fast inactivation by nearly 20 mV is required to produce a comparable shift in the resting potential (Cannon, unpublished observations).

The second gain-of-function defect is an impairment of slow inactivation. With prolonged depolarizations lasting hundreds of milliseconds to seconds, sodium channels become ‘slow inactivated’, as evidenced by the observation that recovery at −100 mV must proceed for several hundreds of milliseconds before the sodium channels become fully available for opening. The two forms of inactivation differ structurally, as well as temporally. Proteolytic digestion of the intracellular face of the channel or mutations of the intracellular loop connecting the third and fourth homologous repeats abolish fast inactivation without disrupting slow inactivation. Given this degree of independence, it was proposed that slow inactivation ought to be disrupted by mutations causing periodic paralysis that may last hours or longer [27]. Otherwise, slow inactivation would shut off the persistent Na current arising from defects of fast inactivation, and the fiber would repolarize within a second or so. In part, this prediction was borne out. Slow inactivation is disrupted for the two most prevalent Na channel mutations in HyperPP (T704M and M1592V) and for another mutation in which the predominant symptom is paralysis, I693T [28,29]. For these mutants the voltage dependence was shifted to depolarized potentials and the maximal extent of slow inactivation was reduced. Of five Na channel mutations associated with myotonia but little or no periodic paralysis, none produced a defect of slow inactivation. On the other hand, some Na channel mutations that consistently produce HyperPP (A1156T, M1360V) or PMC with substantial episodic weakness (T1313M) had normal slow inactivation [29]. Put another way, if slow inactivation is defective then the predominant symptom is always periodic paralysis, not myotonia, but the converse is not true. The interpretation is that defects of slow inactivation predispose the fiber to prolonged episodes of depolarization-induced paralysis, but are not absolutely necessary. Aberrant depolarization and paralysis due to impairment of fast inactivation alone might occur because at this voltage range (−60 to −50 mV) slow inactivation is only about 70% complete in wild-type sodium channels.

One mutant allele of SCN4A associated with HyperPP is due to a double mutation, F1490L/M1493I [30]. The simultaneous occurrence of these missense mutations was identified in two independent HyperPP families. Expression studies in mammalian fibroblasts (HEK cells) did not reveal defects of fast inactivation or activation. The major alteration was an enhancement of slow inactivation, manifest as a 7.5-mV hyperpolarized shift in voltage dependence and slower recovery. The behavior of this mutant suggests that a loss-of-function defect might also be responsible for impaired electrical excitability during an attack of periodic paralysis.

4.2. Hypokalemic periodic paralysis

The molecular defects underlying HypoPP are genetically heterogeneous. The majority of families have missense mutations in *CACNLA3*, whereas missense mutations in *SCN4A* are found in about 10% of families, and in 15% of cases no mutation has been identified [6]. Functional expression studies have identified altered behaviors for both the calcium and sodium channel mutations in HypoPP. However, the mechanisms by which these defects predispose to membrane depolarization and hypokalemia observed during attacks of weakness remains to be established. In vitro studies on biopsied muscle have demon-

strated a consistent physiological defect. HypoPP muscle containing either Ca or Na mutations will paradoxically depolarize in response to hypokalemia whereas normal muscle hyperpolarizes, and this effect is aggravated by insulin [18,31,32].

The L-type calcium channel of skeletal muscle is localized predominantly to the transverse tubular membrane and has a dual role in skeletal muscle [33]. It serves as a voltage-activated Ca^{2+} selective channel and acts as a voltage sensor to trigger Ca^{2+} release from the SR. The latter role is achieved by direct interaction between an intracellular domain of the channel and the calcium release channel (ryanodine receptor) of the SR. The coupling of depolarization to opening of the Ca-release channel is independent of extracellular Ca^{2+} entry. Two lines of evidence suggest that defects in the Ca channel role are critical events in the pathogenesis of HypoPP. First, the final common pathway to episodic weakness is membrane depolarization which could be coupled to Ca^{2+} flux, but is expected to be insensitive to Ca^{2+} release from the SR. Second, measurement of intracellular Ca^{2+} in biopsied fibers with the R528H mutation did not reveal any defect in depolarization-induced Ca^{2+} release from the SR [34].

The functional properties of ionic currents conducted by L-type Ca channels containing HypoPP mutations have been studied in acutely biopsied muscle fibers [34], myotubes cultured from muscle biopsies [35], or in heterologous expression systems [34,36,37]. A consistent finding among all of these studies is that the Ca current density is reduced by about 50% compared to controls. In the one study that compared the behavior of all three Ca channel mutations (Fig. 3) in the same expression system, a common finding was a slowing in the rate of activation [36]. The mutations at R1239 had accelerated rates of closing upon repolarization (fast deactivation) as well. Taken together, all of these functional changes suggest a loss-of-function defect for HypoPP mutations. Two additional lines of evidence support this hypothesis, by failing to demonstrate a gain-of-function defect. First, in vitro experiments on R528H fibers demonstrated that L-type Ca channel blockade (nitrendipine) does not prevent the aberrant depolarization induced by hypokalemic challenge or insulin [32]. Second, a clinical trial with the Ca channel blocker verapamil did not result in clinical improvement [38].

The mechanism by which a loss-of-function defect in the L-type Ca channel predisposes to attacks of depolarization-induced paralysis plus hypokalemia remains to be explained. Two studies on acutely biopsied fibers suggest that Ca channel mutations might somehow be linked to alteration of inward rectifying K currents. Patch-clamp recordings from fibers carrying the R528H mutations demonstrated an impairment of the ATP-sensitive K current [39]. The current amplitude was reduced because of a decrease in the tendency of these channels to open in response to high [Mg-ADP]/[ATP] and because the ability of K^+ ions to pass through the open channel was impaired. Another study using the three-electrode voltage clamp showed a reduction in the membrane conductance of resting HypoPP fibers (-90 to -70 mV range) to K^+, suggesting a defect in an inward rectifying K channel [32]. The notion of a reduced K conductance being the important membrane defect in the pathogenesis of HypoPP is theoretically appealing. Reduction of a cell's permeability to K^+ will depolarize the resting potential and might lead to myoplasmic K^+ accumulation and thereby cause hypokalemia. Indeed, partial block of K permeability in skeletal muscle by Ba^{2+} produces hypokalemia and flaccid paralysis with depolarization [40]. The mechanistic link between the R528H Ca channel mutation and the alterations of inward rectifying K currents, and whether this effect also occurs with the R1239 mutations, remains to be elucidated.

The recent discovery of sodium channel mutations as another cause of HypoPP may provide new insights into the pathomechanism of this disorder. An important consideration in this analysis is the similarity of the clinical phenotype resulting from mutations in *CACNL3A* or *SCNA4*. Mutations in either gene can produce a syndrome with episodic weakness in association with hypokalemia, improvement with K^+ administration, and an absence of myotonia. In vitro electrophysiological behavior is similar as well. Microelectrode recordings from fibers with the sodium channel R672G mutation demonstrated paradoxical depolarization in response to a hypokalemic challenge with 1 mM K^+ [18], just like HypoPP fibers with the Ca channel R528H mutation [31]. The aberrant depolarization was not blocked by tetrodotoxin, which implies that the depolarization was not due to an anomalous inward Na current. Heterologous expression studies of Na channel mutations found in HypoPP have identified an enhancement of inactivation. The two mutations at R672 shifted the voltage dependence of fast inactivation by about 10 mV toward more hyperpolarized potentials [18]. Slow inactivation was enhanced as well [41], due either to a hyperpolarized shift in voltage dependence (R669H, R672G) or a reduced steepness to the voltage dependence (R672H). All of these defects would reduce the fraction of available Na channels (i.e. not inactivated) at the resting potential and increase the tendency for inactivation for mild depolarizations from the resting potential. Thus unlike the mechanism for HyperPP, the Na channel defects resulting in HypoPP are loss-of-function defects. Consistent with a Na channel loss-of-function defect, the peak rate of rise for the action potential and the conduction velocity are both reduced, even when the fiber is artificially hyperpolarized [18]. On the other hand, a loss-of-function defect does not explain why fibers are depolarized during an attack or the basis for hypokalemia with a shift of K^+ into the myoplasm.

4.3. Andersen's syndrome

Nine different mutations (seven missense, two in-frame deletion) in an inward rectifying K channel, Kir2.1, have

been identified in families with AS (Fig. 4). An inwardly rectifying K current is prominent in skeletal muscle: hyperpolarization below the reversal potential for K^+ elicits large inward (negative) K currents, whereas the conductance passes very little current at depolarized potentials. The inward rectifying K current is believed to be important for setting the resting potential of skeletal muscle and for providing an important mechanism for reuptake of K^+ from the transverse tubules. Several inward rectifying K channels are expressed in skeletal muscle (Kir2.1, Kir2.2, Kir2.4), as well as ATP-sensitive K channels that have inward rectifying behavior. The relative contribution of each channel type in the generation of the macroscopic inward rectifying K current is not established.

The functional properties of two AS missense mutations, D71V and R218W, have been assayed by injection of RNA into *Xenopus* oocytes [19]. When either mutant is expressed alone, an inward rectifying K current is not detectable. Mutant subunits are not able to form functional homomultimeric channels. Both mutant transcripts were expressed, however, as shown by co-injection of mutant and wild-type RNA. The co-injection experiments produced a dominant-negative effect: the inward rectifying K currents were smaller than those observed from injecting wild-type Kir2.1 RNA alone. The D71V mutant was particularly potent, with the current amplitude in co-injected eggs being 20-fold smaller than eggs injected with wild-type RNA alone. Other AS mutations in Kir2.1 probably exert dominant-negative effects, but this has not yet been shown experimentally.

Loss-of-function defects in Kir2.1 could account for the triad of periodic paralysis, cardiac arrhythmia, and dysmorphic features in AS. A reduction of the inward rectifying K current in skeletal muscle is expected to predispose the fiber to depolarization (either directly by reducing the K-selective component of the total membrane conductance or indirectly from enhanced K^+ trapping in the transverse tubules). Depolarization will inactivate sodium channels thereby rendering the fiber electrically inexcitable. The Kir2.1 channel is an important contributor to the cardiac inward rectifier current, I_{K1} [42]. This current critically adjusts the timing of cardiac myocyte repolarization to terminate the plateau depolarization. A reduction in I_{K1} prolongs the cardiac action potential, which is evident as a prolonged QT interval on the EKG and an increased susceptibility to ventricular tachyarrhythmias. Targeted disruption of Kir2.1 in knockout mice results in neonatal lethality due to a severe cleft palate and narrowing of the maxilla [43]. Thus although the mechanism is not yet clear, there is precedent for Kir2.1 having an important role in skeletal development which explains the dysmorphic features in AS.

5. Prospectus: genotype or phenotype classification system and implications for therapy

This is a period of rapid discovery in the study of non-dystrophic myotonia and periodic paralysis. Clinical descriptions for many of these disorders have been in the literature for more than a century, whereas, the discovery of the underlying gene defects and the exploration of their consequences is barely a decade old. Not unexpectedly, this rapid progress has brought some confusion along with its wonderful insights into the biology of muscle excitability. Recent progress has highlighted the genetic variability of these disorders: mutations of the same channel gene can produce divergent clinical syndromes (phenotypic heterogeneity), and a well-defined clinical phenotype may arise from mutations of different channel genes (genetic heterogeneity). How then, should the field proceed, with a genocentric or phenocentric view? Such extremist views rarely suffice, and a more balanced approach seems in order.

The clinical delineation of HyperPP and HypoPP remains of paramount importance for the management of patients. It is the patient's response to dietary K^+ or carbohydrate and the serum $[K^+]$ at the onset of an attack that dictate the treatment strategy, not which amino acid residue is mutated. Moreover, comprehensive molecular genetic screening is still not available as a commercial clinical service. These points illustrate the need for retaining a clinical-based classification system for the periodic paralyses.

On the other hand, molecular genetics will play an increasingly important role. These tools have already shown that it is not worth expending clinical effort over the distinction between normokalemic and HyperPP. Both syndromes are part of a spectrum of phenotypes produced by mutations in the sodium channel (*SCNA4*). Conversely, genetic analysis has revealed important distinctions that were not anticipated on clinical grounds. The subset of HypoPP families with Na channel mutations instead of the more commonly occurring Ca channel mutations was not anticipated from the clinical symptoms or response to therapy. Retrospective analysis based on the genetic distinction, however, revealed a potentially important difference in response to therapy. Acetazolamide had a deleterious effect on the frequency and severity of paralytic attacks in HypopPP patients with the R672G sodium channel mutation. The diagnostic precision afforded by genetic screening will sharpen our ability to segregate patients and thereby establish more uniform clinical groups for defining the natural history of a disorder or for assessing the response to therapeutic intervention.

Mechanistic studies on the pathophysiological basis of the symptoms produced by channel gene defects are just beginning. Virtually all of the investigations to date have been limited to an examination of the ability of mutant channels to conduct ionic current. Relatively little work has been done on the cellular biological ramifications. What are the relative levels and turnover rates of mutant and wild-type transcripts? What is the fate of mutant channel proteins in terms of folding, glycosylation, membrane targeting, and oligomerization? Finally, how do mutant channels function in a skeletal muscle environment with

its specialized second messenger systems and signaling pathways? These additional aspects of channel behavior must be addressed to provide the necessary tools for exploring the complexity of behaviors observed in patients, such as environmental triggers or phenotypic differences between patients harboring the same mutation.

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