

TOPICAL REVIEW

Voltage-sensor mutations in channelopathies of skeletal muscle

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Mutations of voltage-gated ion channels cause several channelopathies of skeletal muscle, which present clinically with myotonia, periodic paralysis, or a combination of both. Expression studies have revealed both loss-of-function and gain-of-function defects for the currents passed by mutant channels. In many cases, these functional changes could be mechanistically linked to the defects of fibre excitability underlying myotonia or periodic paralysis. One remaining enigma was the basis for depolarization-induced weakness in hypokalaemic periodic paralysis (HypoPP) arising from mutations in either sodium or calcium channels. Curiously, 14 of 15 HypoPP mutations are at arginines in S4 voltage sensors, and recent observations show that these substitutions support an alternative pathway for ion conduction, the gating pore, that may be the source of the aberrant depolarization during an attack of paralysis.

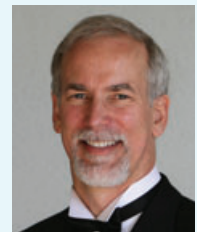
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Derangements of ion channel function are firmly established as the cause of human disease and disability (Lehmann-Horn & Jurkat-Rott, 1999; Ashcroft, 2000). These disorders may be acquired, as with autoantibodies against the nicotinic acetylcholine receptor in myasthenia gravis, or inherited channelopathies such as susceptibility to arrhythmia in long QT Type 3 with mutation of the cardiac sodium channel $\text{Na}_v1.5$. Mutations of genes encoding voltage-gated ion channels expressed in skeletal muscle disrupt the electrical excitability of the sarcolemma and produce two main phenotypes, myotonia and periodic paralysis (Cannon, 2006). Myotonia is a consequence of enhanced sarcolemmal excitability wherein sustained bursts of after-discharges persist for seconds beyond the termination of synaptic input at the neuromuscular junction. These after-discharges trigger sustained Ca^{2+} release from the sarcoplasmic reticulum to produce an involuntary after-contraction that patients describe as stiffness. Conversely, reduced sarcolemmal excitability, which may progress to a complete failure of spike initiation and propagation at the endplate, may cause weakness or a total loss of contraction in an attack of periodic paralysis. A characteristic feature for these channelopathies of skeletal muscle is that the symptoms fluctuate over time, and may be strongly influenced by environmental factors such as temperature, carbohydrate or salt content of food, stress, or prior level of physical activity (Lehmann-Horn *et al.* 2004).

Much has been learned about the pathophysiological basis for the derangements in sarcolemmal excitability that underlie myotonia and periodic paralysis (Lehmann-Horn *et al.* 2004; Cannon, 2006). Heterologous expression studies of mutant ion channels (Cannon & Strittmatter, 1993; Cummins *et al.* 1993; Yang *et al.* 1994), computational simulations of muscle excitability (Cannon *et al.* 1993), pharmacological and genetically engineered animal models (Hayward *et al.* 2008), and microelectrode studies in biopsied human fibres (Lehmann-Horn *et al.* 1981; Rüdél *et al.* 1984) have revealed several common mechanistic themes for these channelopathies of skeletal muscle. Susceptibility to myotonia occurs in two contexts. A marked reduction of the resting Cl^- conductance (Adrian & Bryant, 1974) from loss-of-function mutations

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in the major sarcolemmal chloride channel ClC-1 (Koch *et al.* 1992; Kubisch *et al.* 1998; Pusch, 2002) impairs repolarization after a spike (E_{Cl} approximately equals V_{rest}) and increases the after-depolarization produced by the egress of myoplasmic K^+ into the transverse tubule (loss of Cl^- current to counteract the depolarized shift of E_K). Both effects tend to depolarize the fibre and trigger after-discharges. The second mechanism for eliciting myotonia involves gain-of-function changes for the skeletal muscle sodium channel $Na_V1.4$ (Lehmann-Horn *et al.* 1981) through either disruption of inactivation (Cannon *et al.* 1991) or a hyperpolarized shift of activation (Cummins *et al.* 1993), both of which increase the propensity for sustained bursts of discharges. Several variants of periodic paralysis have been delineated clinically, and all share the common mechanistic feature that during an attack the membrane is aberrantly depolarized and refractory from firing action potentials. In some cases the source of the depolarized shift of V_{rest} was readily apparent: loss of the inward rectifier Kir2.1 in Andersen-Tawil syndrome (Plaster *et al.* 2001; Davies *et al.* 2005) or persistent Na^+ currents (Lehmann-Horn *et al.* 1981; Cannon *et al.* 1991) from impairment of fast- and slow-inactivation of $Na_V1.4$ in hyperkalaemic periodic paralysis (HyperPP). One notable exception had previously defied explanation. In hypokalaemic periodic paralysis (HypoPP) caused by missense mutations in either $Ca_V1.1$ (Ptacek *et al.* 1994) or $Na_V1.4$ (Bulman *et al.* 1999), the aberrant depolarization recorded from biopsied muscle fibres was not prevented by nitrendipine or TTX (Ruff, 1999), and therefore the origin of the depolarizing current remained unknown. The key to resolving this mystery came from the recognition that 14 of 15 HypoPP mutations occur at arginine residues in S4 voltage sensor domains (Matthews *et al.* 2009), coupled with the new observation from structure–function-based studies that mutations at positively charged arginines in S4 may create an alternative conduction pathway for small amplitude gating pore currents (Starace & Bezannila, 2001), also referred to as the ω current (Tombola *et al.* 2005).

Channelopathies often disrupt gating

Now two decades into the molecular era of studying channelopathies, we have learned that a surprisingly large number of mutant alleles do code for functional channels, either as heteromeric protein complexes with wild-type subunits or as monomeric pore-forming proteins (Lehmann-Horn & Jurkat-Rott, 1999). Moreover, the gating behaviour of mutant channels is commonly affected, whereas isolated defects of permeation are exceedingly rare and have not been described for skeletal muscle channelopathies. The other commonly

observed defects for disease-associated mutations are failure to form functional homomeric mutant channels or dominant-negative suppression observed from co-expression with wild-type. The full range of channel defects encompasses several other mechanisms, many of which are not optimally assessed by voltage-clamp recording in artificial heterologous systems. For example, modulation of channel activity, targeting to specialized membrane compartments, and surface expression level may not be faithfully reproduced in heterologous expression systems.

Gating defects have been identified for every $Na_V1.4$ mutant channel associated with myotonia or HyperPP (over 35 tested, see Cannon (2006) for review). In most cases, $Na_V1.4$ mutations produce gain-of-function defects, characterized by impaired inactivation (slowed rate of onset, enhanced rate of recovery, less complete entry, or depolarized shift in voltage dependence) or an enhancement of activation (hyperpolarized shift in voltage dependence or slower rate of deactivation). Channel mutations that alter steady-state gating properties, and thereby cause aberrant persistent inward Na^+ currents, predispose to prolonged attacks of depolarization-induced paralysis. On the other hand, defects in gating kinetics alone, such as a slower rate of inactivation, produce a dynamic instability in membrane potential with susceptibility to myotonia but do not cause paralysis. Many of the $Na_V1.4$ mutations associated with gating defects are located in channel domains that have established contributions to gating, including S4 segments, the domain III–IV linker thought to form the inactivation gate, and the cytoplasmic ends of S5 or S6 segments which may form the receptor for the inactivation gate.

The S4 voltage sensor is a common site of missense mutations

Transmembrane segments and specialized domains within cytoplasmic loops or termini are common sites for disease-associated missense mutations in voltage-gated ion channels. The consensus is that this association occurs because these areas are critical for channel function, whereas amino acid substitutions or in-frame deletions are better tolerated in hydrophilic segments that extend into the aqueous phase and therefore are more likely to be benign polymorphisms. This pattern is distinctly different from the spatial distribution of mutations in disorders for which nonsense or splice site mutations destroy the coding potential of the channel protein. For example, the $Na_V1.1$ nonsense/stop mutations in epilepsy syndromes have a nearly uniform distribution in the primary sequence (114 of over 300 mutations reported; Lossin, 2009). From a total of 53 missense mutations reported in $Na_V1.4$, 21 are in S4 voltage sensors,

whereas 6 of 7 mutations in $\text{Ca}_v1.1$ are in S4 segments. Missense mutations within the pore-loops have not been reported for $\text{Na}_v1.4$ or $\text{Ca}_v1.1$, which is consistent with the experimental observation that gating, rather than permeation, is commonly affected by disease-associated channelopathies. Pore-loop mutations of Kir2.1 have been reported in one variant of periodic paralysis, the Andersen-Tawil syndrome (Plaster *et al.* 2001). These mutant subunits do not form functional Kir channels as homomultimers and exert a dominant-negative effect when co-expressed with wild-type Kir2.1 (Tristani-Firouzi *et al.* 2002).

For the $\text{Na}_v1.4$ sodium channel, a cluster of nine missense mutations occurs in the IVS4 voltage sensor, and five lie in the outermost arginine at the R1 position, R1448C/H/L/P/S. All nine of these missense mutations produce the same clinical phenotype, paramyotonia congenita (PMC), which presents with myotonic stiffness that is aggravated by cooling and paradoxically worsens with repetitive muscle activity (paramyotonia). These PMC mutations all share a common biophysical profile as well, wherein the most pronounced defect observed in voltage-clamp studies of heterologously expressed channels is a 3- to 5-fold slower rate of inactivation (Chahine *et al.* 1994; Yang *et al.* 1994). These observations for disease-associated $\text{Na}_v1.4$ mutations are consistent with biophysical studies using fluorophore-tagged S4 segments (Cha *et al.* 1999) or toxin-modified channels (Sheets & Hanck, 1995) to show that displacement of the voltage sensor in domain IV is tightly coupled to inactivation kinetics, whereas displacement of the voltage sensors in domains I–III is linked to channel activation.

Gating pore currents from S4 mutations in hypokalaemic periodic paralysis

A new class of channel defect was recently discovered for HypoPP mutations in IIS4 of $\text{Na}_v1.4$ (Sokolov *et al.* 2007; Struyk & Cannon, 2007). Historically, the first HypoPP mutations were identified in the α subunit of the skeletal muscle L-type Ca^{2+} channel $\text{Ca}_v1.1$. Curiously, all three mutations were in arginine residues of S4 segments in domains II and IV (Jurkat-Rott *et al.* 1994; Ptacek *et al.* 1994). This discovery generated a series of investigations on the voltage dependence of Ca^{2+} currents and on gating charge displacement (Lapie *et al.* 1996; Jurkat-Rott *et al.* 1998; Morrill & Cannon, 1999). Although some consistent changes were observed (slower activation and reduced current density), these defects could not readily account for the depolarization during an attack of HypoPP. Many years later, several HypoPP mutations were identified in $\text{Na}_v1.4$ (Bulman *et al.* 1999; Davies *et al.* 2001; Sternberg *et al.* 2001; Matthews *et al.* 2009). This observation shattered the hypothesis that HypoPP is exclusively caused

by mutations in $\text{Ca}_v1.1$ while mutations of $\text{Na}_v1.4$ cause weakness in HyperPP or PMC. Moreover, the $\text{Na}_v1.4$ defects in HypoPP were all missense mutations at arginines in S4 voltage sensors. In total, 15 missense mutations have been identified in families with HypoPP: 8 mutations in $\text{Na}_v1.4$ are all at arginines in S4 of domains I, II, or III; 6 mutations in $\text{Ca}_v1.1$ are at arginines in S4 of domain II, III, or IV (see Fig. 1). The final mutation is a V876E in IIS3 of $\text{Ca}_v1.1$, which segregated with HypoPP in a large multi-generation pedigree of a family from South Africa (Ke *et al.* 2009).

The striking homology of the HypoPP mutations in $\text{Ca}_v1.1$ and $\text{Na}_v1.4$ suggested there may be a common functional defect, specific to substitutions at arginines in S4 segments, that may explain why mutations in two voltage-gated ion channels with very different physiological roles would produce a common clinical phenotype. The clue for where to look came from recent studies aimed at determining the conformational changes in S4 during gating. Histidine scanning mutagenesis of S4 in *Shaker* K^+ channels was used as a tool to monitor gating-dependent accessibility of arginines to the intracellular or extracellular solutions, by measuring pH-dependent changes in gating charge displacement (Starace *et al.* 1997). Unexpectedly, these R→H mutants also supported ionic current carried by protons. Mutation of the outermost arginine at position R362 (R1H) created a proton-selective pore that was open at hyperpolarized potentials when the S4 was in the resting conformation 'inward' (Starace & Bezanilla, 2004), whereas the R4H replacement produced a gating pore that passed proton currents at depolarized potentials where S4 shifts to the activated conformation 'outward' (Starace & Bezanilla, 2001). A conceptual model has emerged where, in response to voltage changes, S4 translocates through a narrow crevasse or 'gating pore' of the channel complex. This pore has wide openings at either end, and a short waist, such that aqueous accessibility to S4 arginines shifts from one side of the membrane to the other in response to voltage (Yang *et al.* 1996). Conduction of ions through the gating pore occurs when residues of S4, or the crevasse-lining counterparts (Tombola *et al.* 2005), are mutated. The unitary conductance to protons was quite small, about 40 fS in a gradient of pH_o 5/pH_i 9.2 based on mean-variance analysis (Starace & Bezanilla, 2004). Conduction of protons along the S4 pathway, without a conventional pore domain, was subsequently identified as an intrinsic mechanism of permeation for the voltage-gated proton-selective H_v1 channel family (Ramsey *et al.* 2006; Sasaki *et al.* 2006). Replacement of R1 in the *Shaker* K^+ channel by small hydrophobic residues (R1A/C/S/V) opened a conduction pathway at negative potentials that was permeable to alkali metal cations ($\text{Cs}^+ > \text{K}^+ > \text{Li}^+$) and even guanadinium (Tombola *et al.* 2005). As with the proton currents for R1H channels, the unitary conductance was very small.

For *Shaker* R1C the gating pore current at -200 mV was about 6% of the ' α ' current through the conventional pore at $+50$ mV (Tombola *et al.* 2005). Assuming an open probability of 1 for each conduction pathway at these voltage extremes, and a linear open-channel $I-V$, then the unitary conductance of the gating pore is about 2% of that for the α pore. Considering that there are four R1C subunits in these homotetrameric channels, then the unitary conductance for each R1C is about 0.5% of the conductance for the α pore. The voltage-gated sodium channel is also capable of producing gating pore currents. Glutamine scanning mutagenesis of IIS4 in the rat brain sodium channel $\text{Na}_v1.2$ showed that paired mutations at R1Q/R2Q resulted in gating pore currents activated by hyperpolarization, and the R2Q/R3Q pair was associated with gating pore currents activated by depolarization (Sokolov *et al.* 2005). Finally, a robust naturally occurring gating pore current has been identified in the flatworm $\text{K}_v3.2$ channel, which contains the atypical S4 sequence

with histidine at the R1 position and glycine at R3 (Klassen *et al.* 2008).

HypoPP mutations in S4 of $\text{Na}_v1.4$ result in gating pore currents that are not detectable in wild-type channels (Fig. 2). All five HypoPP mutations in IIS4 have been screened by expression studies in oocytes and produced gating pore currents activated at hyperpolarized potentials (Sokolov *et al.* 2007; Struyk & Cannon, 2007; Struyk *et al.* 2008). The gating pore currents from IIS4-R1H and IIS4-R2H HypoPP mutations were carried exclusively by protons, whereas the gating pores produced by non-histidine mutations at IIS4-R2G/C/S were permeable to alkali metal cations ($\text{K}^+ \approx \text{Cs}^+ > \text{Na}^+ \approx \text{Li}^+$) and even guanadinium. The IIS4-R2Q HypoPP mutation also supports a gating pore conductance active at hyperpolarized potentials and poorly selective among monovalent cations (Francis *et al.* 2010). Notably, the IVS4-R1C mutation associated with PMC does not give rise to detectable gating pore currents (*ibid*). These

	Gating Pore Current	$\text{Na}_v1.4$				Gating Pore Current	$\text{Ca}_v1.1$				Gating Pore Current	<i>Shaker</i> K^+	
		I	II	III	IV		I	II	III	IV			
R0		S	S	K	T		K	S	K	A		A	
		A	V	S	L		A	V	I	F		I	
		L	L	L	F		L	L	L	F		L	
R1	hyperpol	R	R	R	R	?	R	R	R	R		hyperpol	R
		T	S	T	V		A	C	V	L		V	
		F	F	L	I		F	I	L	F		I	
R2	hyperpol	R	R	R	R	?	R	R	R	R		xporter	R
		V	L	A	L		V	L	V	V		L	
		L	L	L	A		L	L	L	M		V	
R3	depol	R	R	R	R	?	R	R	R	R		xporter	R
		A	V	P	I		P	I	P	L		V	
		L	F	L	G		L	F	L	I		F	
R4		K	K	R	R		R	K	R	K		depol	R
		T	L	A	V		L	I	A	L		I	
		I	A	L	L		V	T	I	L		F	
R5		T	K	S	R		S	K	N	S		K	
		V	S	R	L		G	Y	R	R		L	
		I	W	F	I		V	W	A	A		S	
R6		P	P	E	R		P	T	K	E		R	
		G	T	G	G		S	S	G	G		H	
		L	L	M	A		L	L	L	V		S	
R7		K	N	R	K		Q	S	K	R		K	

Figure 1. Alignment of S4 sequences for $\text{Na}_v1.4$, $\text{Ca}_v1.1$ and *Shaker* K^+ channels

Positively charged residues are delineated with a shaded background (blue) for the R0 to R7 position. Mutations of these basic residues may produce a gating pore current activated by hyperpolarization (hyperpol), by depolarization (depol), or give rise to a proton transporter (xporter). The gating pore waist lies between R2 and R3, and voltage-dependent translocation of substituted R residues on S4 through this region regulates the flow of gating pore current. HypoPP mutations (red) are clustered at arginines in the R1 or R2 position of $\text{Na}_v1.4$ and $\text{Ca}_v1.1$. Mutations of $\text{Na}_v1.4$ at IV-R1 (green) are associated with PMC, not HypoPP, and do not produce gating pore current. A mixed variant of periodic paralysis is associated with mutations at $\text{Na}_v1.4$ II-R3 (yellow) which causes a depolarization-activated gating pore.

observations provide additional evidence that the four voltage-sensor domains in Na_v channels are not equivalent and support the notion that an anomalous gating pore current is necessary and sufficient to cause HypoPP. Heterologous expression of $\text{Ca}_v1.1$ in non-muscle cells is poor, and this technical challenge has impeded the ability to test with sufficient sensitivity for gating pore currents from HypoPP mutations in the calcium channel. An unusual variant of periodic paralysis has been associated with missense mutations of $\text{Na}_v1.4$ at IIS4-R3G/Q/W (Vicart *et al.* 2004). The clinical phenotype has features of HyperPP and normokalaemic periodic paralysis, but is clearly distinct from HypoPP. Heterologous expression studies show all three mutations produce gating pore currents, but the conductance activates with depolarization greater than -40 mV (Sokolov *et al.* 2008). Interestingly, mutagenesis studies with IIS4-R3Q in $\text{Na}_v1.2$ did not produce a gating pore current (Sokolov *et al.* 2005). Only the double mutant $\text{Na}_v1.2$ IIS4-R2Q/R3Q produced a gating pore current, which demonstrates isoform-specific differences in the ability to conduct gating pore currents in the Na_v family.

Implications of a gating pore current for muscle fibre excitability

The identification of gating pore currents in association with HypoPP is a major advancement, but the impact of this discovery hinges upon establishing a mechanistic link between this unusual current and susceptibility to depolarization-induced attacks of weakness in the setting of low extracellular K^+ . One obvious consideration is the relatively small amplitude of gating pore currents. To address this question, it is crucial to estimate the gating pore current density in muscle fibres, and consensus is lacking on this point. Sokolov *et al.* (2007) estimate that the gating pore current, I_{gp} , is about 1% of the central pore Na^+ current, I_{Na} , based on favourable recordings where the expression level was well-matched to measure both I_{Na} and I_{gp} after TTX application in the same egg. We

estimated a 50-fold smaller I_{gp} , about 0.02% of I_{Na} , based on the ratio of I_{gp} to total gating charge displacement and the assumption of 12 charges per channel (Struyk *et al.* 2008). The peak Na^+ conductance in mammalian skeletal muscle is ~ 50 mS cm^{-2} , and so in the heterozygous state, the predicted gating pore conductance at the resting potential is $5 \mu\text{S cm}^{-2}$ or $\sim 1\%$ of the total membrane resting conductance. Under physiological conditions at rest, a steady inward current of $\sim 0.5 \mu\text{A cm}^{-2}$ would flow, carried by protons for R \rightarrow H mutant channels and by Na^+ for all other missense R/X mutations. Curiously, the HypoPP clinical phenotype is nearly indistinguishable for R \rightarrow H mutations compared to other substitutions (Sternberg *et al.* 2001). One hypothesis for the basis of this similarity, despite very different charge carriers, is that the Na^+/H^+ exchanger effectively converts a proton leak to a Na^+ leak (Jurkat-Rott & Lehmann-Horn, 2007).

The mechanism by which the relatively small-amplitude I_{gp} produces susceptibility to large depolarized shifts of V_{rest} in hypokalaemia with a loss of fibre excitability has been linked to the balance between the inward I_{gp} and the small outward K^+ current passed by the inward rectifier I_{Kir} under resting conditions (Struyk & Cannon, 2008). The phenomenon depends critically on the non-linear I - V characteristics of I_{Kir} . Indeed, an approximation based on ohmic behaviour predicts only a small depolarization of V_{rest} by I_{gp} of magnitude: $\Delta V_{\text{rest}} = (0.5 \mu\text{A cm}^{-2}) / (200 \mu\text{S cm}^{-2}) = 2.5$ mV. (A conductance of $200 \mu\text{S cm}^{-2}$, or about 20% of the total resting conductance of a muscle fibre, is used for this calculation because Cl^- is passively distributed in the steady state, and therefore the large Cl^- conductance does not contribute to setting V_{rest} .) The non-linear behaviour of the steady-state I - V relation of mammalian skeletal muscle is shown by the simulated response in Fig. 3, which consists of three components, $I_{\text{mem}} = I_{\text{Kir}} + I_{\text{KDR}} + I_{\text{Leak}}$, where I_{KDR} is the delayed rectifier and I_{Leak} is a small ohmic conductance with a reversal potential of 0 mV. Although the Cl^- conductance accounts for $\sim 80\%$ of the total membrane conductance of

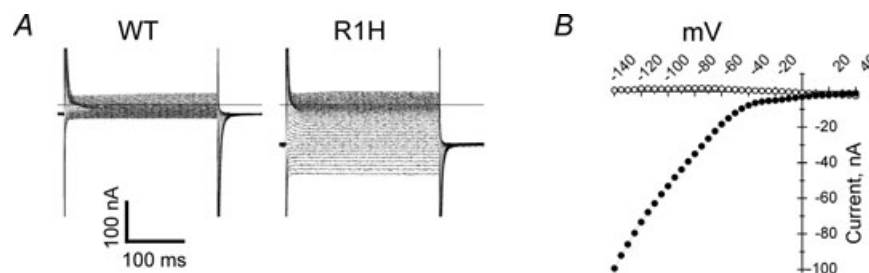


Figure 2. Hyperpolarization-activated gating pore current in the R1H HypoPP mutation of $\text{Na}_v1.4$

A, currents elicited by 300 ms step depolarizations between -140 mV and $+30$ mV from a holding potential of -100 mV in oocytes expressing wild-type (WT) or R1H channels. Saturating concentration of TTX has been added to suppress I_{Na} . B, steady-state I - V relation, after subtraction of linear background leak: WT, open circles; R1H, filled circles. Modified from Struyk & Cannon (2007) with permission.

muscle at rest, and on a rapid time scale of milliseconds is very important for repolarization and prevention of myotonic after-discharges, it has almost no influence on the steady-state I - V relation because of passive redistribution of Cl^- . As a result, V_{rest} is determined by a balance of two relatively small currents, the outward component of the I_{Kir} and the inward I_{Leak} . A reduction in extracellular K^+ will not only cause a hyperpolarized shift of E_{K} , but also produces a leftward shift of the outward 'hump' of I_{Kir} . Under extreme conditions, such as $[\text{K}^+]_o$ of ~ 1 mM, the outward hump of I_{Kir} is overwhelmed at all voltages

by the inward I_{Leak} . V_{rest} will now be set by a balance between I_{Leak} and I_{KDR} . Moreover, the outward current from I_{KDR} becomes substantial only for $V > -55$ mV and therefore the balance point between I_{Leak} and I_{KDR} is determined primarily by the voltage at which the K_{DR} channel activates (i.e. by gating behaviour, rather than by reversal potential). The end result is an apparent decoupling of V_{rest} from E_{K} to produce the paradoxical depolarization of V_{rest} in low external K^+ that has been observed for cells in which I_{Kir} is the predominant K^+ conductance for normally setting V_{rest} , as in skeletal

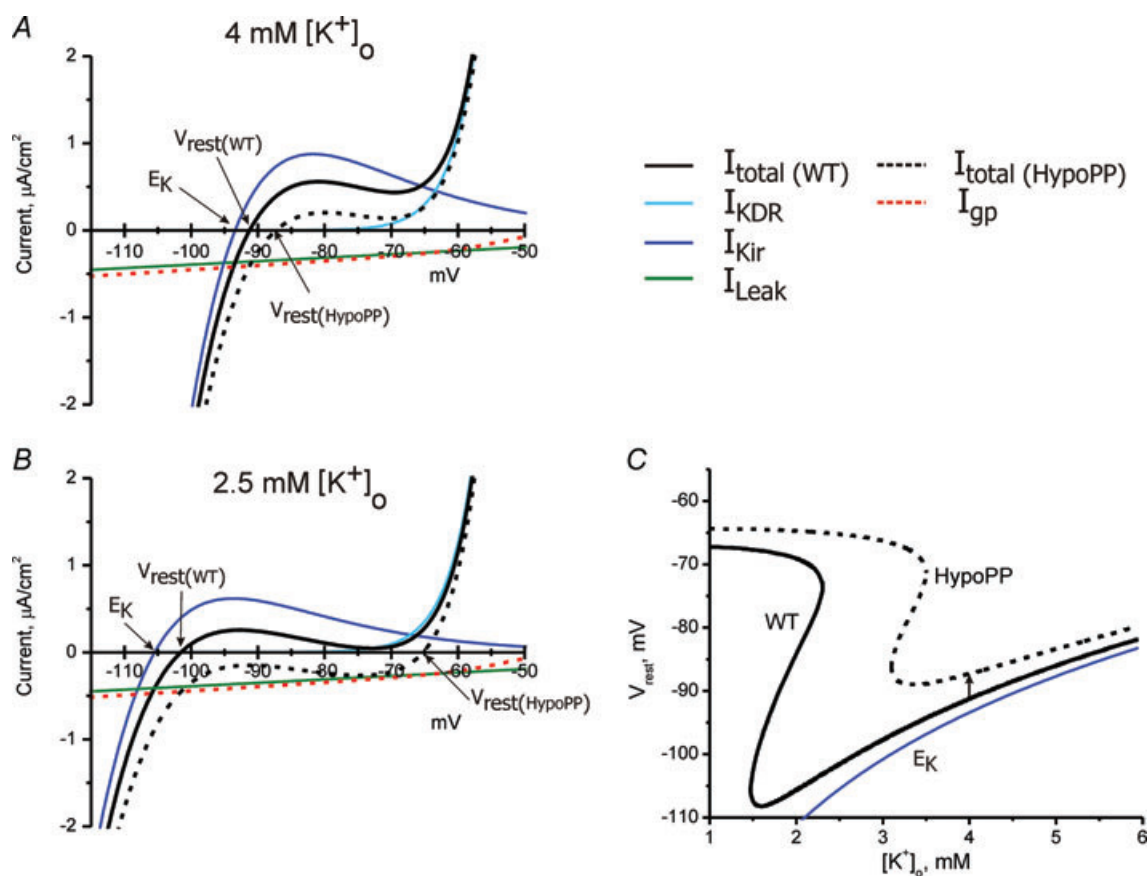


Figure 3. The gating pore current increases the susceptibility to paradoxical depolarization in low extracellular $[\text{K}^+]$

The steady-state I - V relation for mammalian skeletal muscle was simulated by the combination of an inward rectifier K^+ current, I_{Kir} , a delayed rectifier K^+ current, I_{KDR} , and a leakage current with a reversal potential of 0 mV, I_{Leak} , as in Struyk & Cannon (2008). **A**, in $4 \text{ mM } [\text{K}^+]_o$ the resting potential of -91.3 mV is determined primarily by the balance of an inward I_{Leak} and outward I_{Kir} . Addition of a gating pore current, I_{gp} , to simulate HypoPP (dashed red line), shifts the I - V relation downward (dashed black line) but results in only a small depolarization of V_{rest} to -87.3 mV . **B**, reduction of $[\text{K}^+]_o$ to 2.5 mM shifts E_{K} and I_{Kir} to more negative potentials, with a predicted hyperpolarization of V_{rest} to -101.6 mV in WT fibres. For HypoPP fibres, however, V_{rest} depolarizes to -65.3 mV (arrow) because the combination of inward currents ($I_{\text{Leak}} + I_{\text{gp}}$) exceeds the outward current from I_{Kir} . Under these conditions, which would result in paralysis from inactivation of sodium channels, V_{rest} is now set by the balance of I_{KDR} and the inward currents. Because K_{DR} channels are active only for $> -55 \text{ mV}$, V_{rest} is strongly dependent on K_{DR} gating, which results in an apparent decoupling of V_{rest} from E_{K} . **C**, phase plot of V_{rest} as a function of $[\text{K}^+]_o$ for simulated WT and HypoPP fibres. The inward gating pore current in HypoPP fibres causes only a small depolarization of V_{rest} from -91.3 mV to -87.3 mV in $4 \text{ mM } [\text{K}^+]_o$ (arrow). More importantly, the catastrophic depolarization of V_{rest} shifts leftward from $1.5 \text{ mM } [\text{K}^+]_o$ for WT to 3 mM for HypoPP. The Nernst potential for K^+ , E_{K} , is shown for comparison (blue line).

muscle (Carmeliet, 1982) and heart (Siegenbeek van Heukelom, 1991). The gating pore produced by HypoPP mutations in arginines of S4 segments has a conductance that is of comparable amplitude to the endogenous I_{Leak} (Fig. 3B). The impact on V_{rest} is that the catastrophic depolarization as external K^+ is lowered now occurs at a higher $[K^+]_o$, around 2.5–3.0 mM instead of ~ 1 mM (Struyk & Cannon, 2008). The phase plot of V_{rest} as a function of $[K^+]_o$ (Fig. 3C, arrow) shows that while I_{gp} causes only a small 4 mV depolarization of V_{rest} in 4.0 mM $[K^+]_o$, I_{gp} induces a critical rightward shift of 1.5 mM in the catastrophic break point such that susceptibility to depolarization now occurs in the physiological range for $[K^+]_o$ fluctuations.

Pharmacological studies support the theoretical notion that a balance between I_{Kir} and $I_{Leak} \pm I_{gp}$ is the critical determinant for the paradoxical depolarization in low K^+ that triggers an attack of HypoPP. Barium toxicity produces a secondary form of hypokalaemic periodic paralysis (Gallant, 1983), and the Kir channel is very sensitive to Ba^{2+} block. Studies in mouse muscle that were designed to detect the paradoxical depolarization as a function of $[K^+]_o$ showed that in response to a 2 mM K^+ challenge normal fibres hyperpolarized by 8 mV whereas fibres in $10 \mu M Ba^{2+}$ (50% block of I_{Kir}) depolarized by 17 mV (Struyk & Cannon, 2008). Reduction of K_{ATP} channel activity has been reported in excised patches from human HypoPP fibres with the $Ca_v1.1$ -R528H mutation (Tricarico *et al.* 1999). Moreover, administration of insulin reduced the outward limb of I_{Kir} in human HypoPP fibres, making them more susceptible to aberrant depolarization (Ruff, 1999). Taken together, these observations suggest that attacks of HypoPP triggered by carbohydrate ingestion may be linked to insulin secretion with the attendant reduction in I_{Kir} specifically from an increased intracellular ratio of $[ATP]/[ADP]$ which would inhibit K_{ATP} channels. A secondary insulin effect would be a shift of extracellular K^+ into the myoplasm from increased activity of the Na^+/K^+ -ATPase. Both effects, decreased K_{ATP} activity and reduced extracellular $[K^+]_o$, would contribute to the development of paradoxical depolarization and weakness. The reciprocal experiment, to increase the inward leak rather than decreasing Kir, also showed a shift of the catastrophic break point of V_{rest} to higher $[K^+]_o$. In control conditions, the paradoxical depolarization occurred in 1.5 mM $[K^+]_o$, whereas a leak produced by the ionophore amphotericin caused the depolarization shift to occur at 2.5 mM (Jurkat-Rott *et al.* 2009).

Conclusions

Channel gating is commonly affected by channelopathies, and the causative mutations often reside in the S1–S4 voltage sensor domain. An additional anomaly, the gating

pore current, has been identified for missense mutations at arginine residues in S4 voltage sensors of the skeletal muscle sodium channel $Na_v1.4$. Several lines of evidence support the hypothesis that the gating pore current plays a causative role in the pathogenesis of HypoPP. All eight HypoPP mutations identified thus far in $Na_v1.4$ are at arginines in the R1 or R2 position. Six of seven HypoPP mutations in $Ca_v1.1$ are at homologous S4 arginines, and the final mutation is in S3, which potentially could also give rise to a gating pore current. Six of the $Na_v1.4$ HypoPP mutations have been tested by functional expression, and all produce gating pore currents activated at hyperpolarized potentials. Moreover, the gating pore hypothesis provides a common mechanism by which mutations of either $Na_v1.4$ or $Ca_v1.1$ cause a common clinical phenotype. Finally, model simulations demonstrate that gating pore currents, although small in amplitude, are sufficient to cause paradoxical depolarization in the setting of mild hypokalaemia. Over 45 missense mutations at arginines in S4 voltage sensors of Na_v , Ca_v and K_v channels have been identified in various channelopathies, and it is likely that gating pore currents may also play a role in epilepsy, migraine and cardiac arrhythmia. The gating pore current also represents an opportunity for new therapeutic strategies. Agents that block the gating pore current, without affecting channel gating, would be ideal drugs to treat these disorders.

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