Do Hyperpolarization-induced Proton Currents Contribute to the Pathogenesis of Hypokalemic Periodic Paralysis, a Voltage Sensor Channelopathy?

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An increasing number of human diseases have been found to result from mutations in ion channels, including voltage-gated cation channels. Though the mutations are known, the pathophysiological mechanisms underlying many of these channelopathies remain unclear. In this issue of the Journal, Struyk and Cannon (see p. 11) provide evidence for a novel mechanism, proton movement catalyzed by the voltage-sensing domain of the mutant channels. It already is known that voltage-gated proton channels resemble the voltage sensor domains of cation channels and show depolarization-induced outward currents and current reversal at the H+ equilibrium potential. It also is well established that voltage-gated K+ channels can conduct or transport protons when specific voltage sensor arginines are replaced by histidines—and that the pathway for the protons may be relevant for the pathogenesis of the disease (hypokalemic periodic paralysis).

Superfamily of Voltage-gated Cation Channels
Voltage-gated cation channels (VCCs) are proteins that conduct Na+, Ca2+, or K+ with high selectivity through a central, so-called α pore. Precise control of channel opening and closing is necessary for proper cell excitability and particularly the generation of action potentials. VCC are characterized by at least one ion-conducting open and two nonconducting states, one from which the channel can be activated (the resting state) and one from which it cannot (the inactivated state). The transition from one state to another is voltage dependent. The function of the channels’ voltage-sensing domains has been extensively characterized.

Generally, VCCs consist of four repeats (I–IV) of domains, consisting of six transmembrane α-helical segments, S1–S6. The voltage-sensing domain is formed by S1–S4 with S4 being the most mobile region—thought to move outward along a helical screw (for review see Lehmann-Horn and Jurkat-Rott, 1999). Because S4 carries a positive amino acid residue at every third position, the S4 movement through the electric field of the membrane generates the so-called gating current even when the α pore is blocked. The depolarization-driven outward movement of S4 drives the conformational change that results in channel activation and conduction of ions through the central α pore formed by S5 and S6 and the intervening linker.

Hyperpolarization-activated Cation Currents through Mutant VCC
In this issue, Struyk and Cannon report that a NaV1.4 sodium channel mutation causing hypokalemic periodic paralysis type 2 leads to a hyperpolarization-activated current, Ih, which is carried by protons. The mutation is located at the extracellular end of the S4 voltage sensor of repeat II and replaces the outmost Arg663 of the rat homologue rNav1.4 by a histidine. We denote this substitution R1H to indicate that it is the first of the S4 arginines that is mutated. At the resting membrane potential, −85 mV, Ih is inward when pHi = pHe = 7.4 and outward when pHi = 5 at unaltered pHe (with an apparent reversal potential close to EiH = −150 mV) (Fig. 1). Ih decreases at potentials more positive than −50 mV and its voltage dependence correlates roughly with the gating currents, suggesting that movement of S4 occludes a proton pathway that is different from the α pore. Because the experiments were done in the presence of the α pore blocker tetrodotoxin, the notion of a non-α pore in the region of S4 was introduced to provide a pathway for Ih. As in other proton channels, the histidine residue was suggested to act as proton acceptor/donor and, thus, mediate the proton current, as previously was suggested for the M2 channel of influenza viruses (Venkataraman et al., 2005).

Due to the homology within the VCC superfamily, the study of Struyk and Cannon (2007) can be compared with experiments with a designed mutation in the Drosophila Kv1 equivalent, Shaker, in which R1H (Arg362His)

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Abbreviations used in this paper: HypoPP, hypokalemic periodic paralysis; VCC, Voltage-gated cation channel.
generates an inward-going proton current $I_h$ that becomes increasingly prominent as the proton reversal potential $E_H$ is shifted to more positive values (Starace and Bezanilla, 2004). However, the Shaker $I_h$ could be blocked (at $pH_7.4$) by mM concentrations of $Ni^{2+}$, a metal cation that interacts with histidines. This is in contrast to the $I_h$ described by Struyk and Cannon, which could not be blocked by several divalent cations, including $Ni^{2+}$. This lack of $Ni^{2+}$ block may reflect a hyperpolarization-induced “inaccessibility” of hNav1.4 R1H, similar to what has been observed for MTS reagents (Yang et al., 1996).

In the Shaker voltage sensor (Starace and Bezanilla, 2004), substitutions of histidine for residues in S1 (I241) and S2 (I287) also causes the appearance of proton currents that can be blocked by mM $Ni^{2+}$ (Campos et al., 2007). As disulfide bridges can form between R1C of the S4 segment and I241C and I287C, these sites seem to be in close vicinity and may contribute toward forming the lining of the pathway for proton movement. Moreover, substitutions at the S2 sites 283, 286, and 290 increase the cation current, suggesting that they line and enlarge the same pathway (Tombola et al., 2005, 2007).

In Shaker, R1 substitutions to residues other than histidine result in an $I_h$ carried by alkali metal cations (Tombola et al., 2005). Whether protons are conducted has not been tested. This current varied with the identity of the substituted residue: R1S > R1C > R1V ~ R1A, had the selectivity $Cs^+ \geq K^+ > Li^+$, was not affected by $\alpha$ pore blockers such as AgTx2, and was called $\omega$ current, to distinguish it from the $\alpha$ pore current. Tombola et al. (2005) also demonstrated that R1H did not conduct cations, suggesting that the histidine residue may obstruct the otherwise relatively large, unselective $\omega$ pathway.

There also have been reports of $I_h$ in mutants of the second arginine of S4. The naturally occurring R2G of rNav1.4 conducted an $I_h$ cation current (Sokolov et al., 2007), whereas no $I_h$ was detectable in R2Q in Na1.2 (Sokolov et al., 2005). In contrast to the aforementioned findings, it was necessary to replace the first and second arginine in Na1.2, to observe an $I_h$ current (Sokolov et al., 2005). This could merely be an additive effect of the two mutants.

**Depolarization-activated Currents through $\omega$ Pathway:**

**Cation Leak or Proton Transport**

In Shaker, R2C (Arg365Cys) exposed no $I_h$ cation current (Tombola et al., 2005), in apparent contrast to the presence of an $I_h$ in R2G of rNav1.4. This difference may reflect different localization (and thus accessibility) of the S4 segments with respect to the intra- and extracellular space. In Nav channels, both R1 and R2 of the second S4 are accessible from outside at the resting membrane potential (Cestele et al., 2001) with R2 also being accessible from inside (Kuzmenkin et al., 2002). In Shaker, only R1 is accessible from the outside at rest (Starace and Bezanilla, 2001), whereas R2 is not, so a depolarization is required to generate a current, $I_d$, through the $\omega$ pathway. Depending on the degree of the depolarization, the voltage sensor will move between discrete positions. If R2 (or R3) is replaced by a histidine and then alternately exposed to the intra- and extracellular space, protons will be transported at depolarized potentials (Starace and Bezanilla, 2004). The magnitude and direction of the current will depend on the $pH_i/pH_o$ difference (Fig. 1).

The Shaker mutants R1H, R2H, and R3H conduct or transport protons at different potential ranges, which
may correspond to the S4 movement during depolarization (Starace et al., 1997, Starace and Bezanilla, 2001). The potential range over which this current is observed corresponds to the voltage window over S4 charges being moved. The current is maximal at potentials close to midpoint of the QON–voltage relation (Fig. 1). No protons are transported when the voltage sensors are immobilized. Similar to R1H, R4H produces a proton leak but at more positive potentials. In contrast to R1 and R4, R5H does not generate any current, suggesting that R5 is not accessible from the inside or the outside or does not move (Starace and Bezanilla, 2001).

Channelopathies due to S4 Mutations

Channelopathies are disorders caused by a change of ion channel function. Most channelopathies are associated with mutations that alter channel state transitions such as activation, inactivation, and recovery from inactivation, which will result in changes in the ionic current through the α pore. In addition to mutations in other functionally important regions, voltage sensor mutations have been detected in many diseases of brain (epilepsy, episodic ataxia, familial hemiplegic migraine), heart (long QT syndromes), and skeletal muscle (hypokalemic periodic paralysis, paramyotonia, potassium-aggravated myotonia) (for review see Lehmann-Horn and Jurkat-Rott, 1999). The disease in which mutations have only been found in voltage sensors are hypokalemic periodic paralysis (HypoPP) types 1 and 2. In both types, episodes of generalized muscle weakness occur occasionally, often during the second half of night after a day characterized by strong exercise. Another trigger is a carbohydrate-rich meal; glucose and released insulin induce a rapid uptake of K⁺ into the muscle fibers. The resulting hypokalemia correlates well with the clinical expression of the paralytic attack and gave the disease its name. If no K⁺ is substituted, the weakness can last several hours or days until the serum level is normalized by a hypokalemia-induced rhabdomyolysis or K⁺ retention. As muscle strength is normal between attacks, at least in young patients, the underlying ion channel defect must be well compensated. In HypoPP-1, mutations have been identified in the Ca,l,1 voltage sensors (S4 segments) of repeats II (R1H/G) or IV (R2H/G) (Jurkat-Rott et al., 1994). The resulting changes in the α pore currents were minor and showed reduced function rather than gain of function. For a dominantly inherited disease such as HypoPP, due to mutations in a monomeric protein, gain of function would be expected rather than haploinsufficiency (for review see Lehmann-Horn and Jurkat-Rott, 1999). Therefore the pathogenesis of the disease remains enigmatic to both clinicians and laboratory investigators. HypoPP-2 mutations are located in S4 segments of Nav1.4 repeats II (R1H/G) or IV (R2H/G) (Jurkat-Rott et al., 1994). The resulting changes in the α pore currents were minor and showed reduced function rather than gain of function. For a dominantly inherited disease such as HypoPP, due to mutations in a monomeric protein, gain of function would be expected rather than haploinsufficiency (for review see Lehmann-Horn and Jurkat-Rott, 1999). Therefore the pathogenesis of the disease remains enigmatic to both clinicians and laboratory investigators. HypoPP-2 mutations are located in S4 segments of Na,l,4 repeats II (R1H, R2H/G/S/C) and III (R3Q, the most lysine is equivalent to an arginine and numbered as 1), and the resulting reduction of channel availability may only partially explain the clinical symptoms (Jurkat-Rott et al., 2000; Struyk et al., 2000).

Pathogenesis of HypoPP Attacks and Significance of Iₜ\text{h}

In vitro measurements on native muscle fibers from a HypoPP-2 patient revealed resting membrane potentials that were 8 mV less negative than in normal controls.
(Jurkat-Rott et al. 2000), in the same range as the slightly depolarized potentials found in HypoPP-1 patients (for reviews see Lehmann-Horn and Jurkat-Rott, 1999; Ruff, 1999). Depending on the mutation, the reason for this as yet unexplained membrane depolarization could be the recently described Na⁺ leak (Sokolov et al., 2007) or the potential-dependent proton current (Struyk and Cannon, 2007) through Nav1.4. As the R2G-induced Na⁺ leak through the ω pathway is larger than the K⁺ conductance, the resulting membrane depolarization should be large although limited by the decrease of the ω pathway–associated leak with depolarization. In contrast, the R1H-induced proton conductance at a small pH gradient is much smaller than the K⁺ conductance at physiological [K⁺]o, and the pathologic proton inward current will be balanced by K⁺ efflux at a slightly less negative membrane potential. So the resulting depolarization should be just 7 mV according to our calculations (from −85 to −78 mV).

In any case, the new electrical steady state will lead to changes in the concentration of various intracellular buffers and activation of several ion transporters (Fig. 2 A). For the proton Iₙ described by Struyk and Cannon, the inward moving protons may be buffered by protein and inorganic buffers (e.g., phosphate), transported out of the cell by the Na⁺/H⁺ exchanger and the lactate/H⁺ cotransporter, and/or neutralized by HCO₃⁻ to form H₂O and CO₂ in a reaction catalyzed by the myoplasmic carbonic anhydrase. The HCO₃⁻ could be supplied by the Na⁺/HCO₃⁻ cotransporter. Taking all mechanisms together, an increased proton influx would lead to a secondary intracellular accumulation of Na⁺ and depletion of lactate. As the Na⁺ leak reported for the R2G mutation (Sokolov et al., 2007) should also lead to an intracellular Na⁺ accumulation, the effects of R1H and R2G on the ion gradients may be qualitatively similar. However the issue of intracellular Na⁺ accumulation has not been unambiguously clarified in vivo: there is one report of elevated muscle Na⁺ content (Engel et al., 1965) and one with a decreased concentration (Niall and Pak Poy, 1966). Future studies are necessary to answer this question.

The intermittent attacks of weakness in HypoPP lead to the requirement of trigger mechanisms. Insulin secretion, as after carbohydrate-rich meals, is one such trigger. Insulin activates the electrogenic Na⁺/K⁺-ATPase; insulin per se and the resulting decrease in [K⁺]o normally lead to a membrane hyperpolarization. In contrast to normal muscle, however, but similar to HypoPP-1, HypoPP-2 muscle fibers depolarize to −50 mV at a reduced [K⁺]o of 1 mM and loose force (Jurkat-Rott et al., 2000). This explains the hypokalemic weakness of the patients. The mechanism underlying this depolarization has not yet been solved. Given the results of Struyk and Cannon, it would be tempting to propose that the hypokalemia initially causes a hyperpolarization, as in normal fibers, and that the ensuing Na,1.4-mediated proton current cannot be balanced by the K⁺ current as it strikingly is reduced at low [K⁺]o. Exceeding the threshold current shown in Fig. 2 B, the fibers would depolarize to −50 mV along a bistable current–voltage relationship characterized by a “negative resistance” (N-shape; Gadsby and Cranefield, 1977). However, the decrease of the proton leak with depolarization should not be neglected, however, as this would tend to dampen the membrane depolarization.

Secondary effects or additional pathophysiological mechanisms may be required to explain the large depolarization that causes the paralysis. One possible such mechanism could be that HypoPP muscle fibers become K⁺ depleted due to the uptake of Na⁺ and that glucose uptake and insulin secretion will shift more K⁺ into the K⁺-depleted fibers than would be the case in controls. The interstitial, and in particular the T-tubular [K⁺], then could become so low that the K⁺ conductance approaches zero and that, despite a very negative K⁺ reversal potential, the low K⁺ conductance severely limits the K⁺ contribution to the resting membrane potential, thereby causing the cell membrane to depolarize, which would inactivate the Na⁺ channels and thereby paralyze the fibers.

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