

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

Karin Jurkat-Rott and Frank Lehmann-Horn

Institute of Applied Physiology, Ulm University, 89069 Ulm, Germany

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 differs from the K^+ conducting pore (Starace et al., 1997). In this issue, Struyk and Cannon show that a mutation in the voltage sensing domain of a voltage-gated Na^+ channel can behave similarly and further raise the question of whether this additional membrane conductance for 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^+ , Ca^{2+} , 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 $Na_v1.4$ sodium channel mutation causing hypokalemic periodic paralysis type 2 leads to a hyperpolarization-activated current, I_h , 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, I_h is inward when $pH_i = pH_o = 7.4$ and outward when $pH_i = 5$ at unaltered pH_o (with an apparent reversal potential close to $E_H = -150$ mV) (Fig. 1). I_h 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 I_h . 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)

Correspondence to Frank Lehmann-Horn:
frank.lehmann-horn@uni.ulm.de

Abbreviations used in this paper: HypoPP, hypokalemic periodic paralysis; VCC, Voltage-gated cation channel.

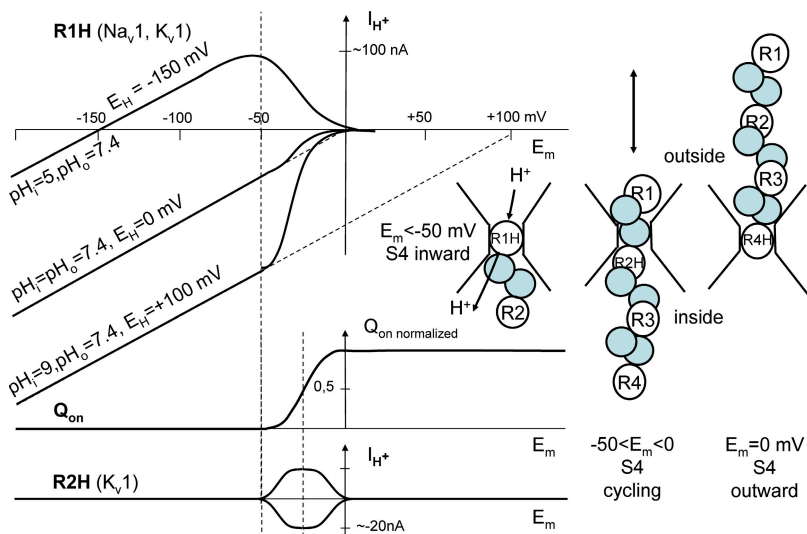


Figure 1. Schematic drawing of proton currents through *Shaker* potassium and Na_v1 sodium channels carrying R-to-H substitutions in S4 voltage sensors (data extrapolated from Struyk and Cannon, 2007). When the outmost arginine is replaced by histidine (R1H) in *Shaker* and Na_v1 , membrane hyperpolarization activates an inward proton current when $\text{pH}_i \geq \text{pH}_o$. Dependent on the membrane potential and the proton reversal potential E_{H^+} , at $\text{pH}_i \ll \text{pH}_o$, the current can be inward or outward. A similar current has been reported in R4H when the S4 segment is in the most outward position. At least in *Shaker* channels, R2H and R3H generate a proton transport by movements between discrete inward and outward positions of the voltage sensor if pH_i and pH_o are different. In any case, at depolarized potentials at which S4 segments have moved, the current approaches zero. The right panels show how the R1H (left) and R4H (right) side chains might obstruct the pathway for Na^+ and K^+ but still conduct H^+ . In the middle of the right panel, R2H can transport protons if alternatingly inward and outward exposed.

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_o 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: $\text{R1S} > \text{R1C} > \text{R1V} \sim \text{R1A}$, had the selectivity $\text{Cs}^+ \geq \text{K}^+ > \text{Li}^+$, was not affected by α pore blockers such as AgTx2, and was called ω current, to distinguish it from the α 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 ω 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 $\text{Na}_v1.2$ (Sokolov et al., 2005). In contrast to the aforementioned findings, it was necessary to replace the first and second arginine in $\text{Na}_v1.2$, 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 ω 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 ω 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 alternatingly exposed to the intra- and the 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

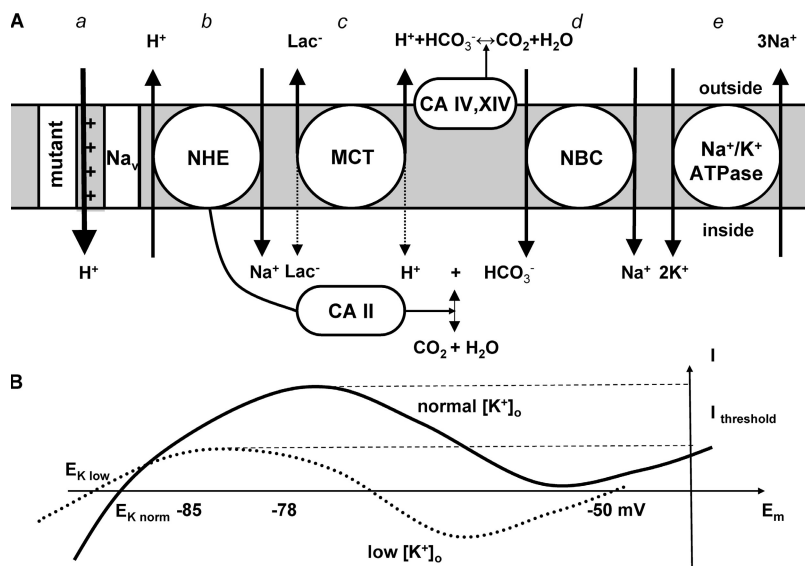


Figure 2. (A) Channel and transporter mediated ion movements between myoplasm and the interstitial fluid and (B) current-voltage relationships of muscle fibers at normal and low $[K^+]_o$. The (a) influx of H^+ through the mutant $Na_v1.4$ channel will lead to compensatory mechanisms at surface and T-tubular membranes: (b) increased efflux of H^+ by the Na^+/H^+ exchanger, NHE; (c) increased efflux of H^+ and Lac^- (lactate⁻) by the monocarboxylate transporter MCT; (d) activation of the Na^+/HCO_3^- cotransporter NBC as $[HCO_3^-]_i$ reacts with protons to form H_2O and CO_2 , a reaction that is catalyzed by the myoplasmic carbonic anhydrase (CA II). These alterations will lead to an intracellular accumulation of Na^+ , which activates the Na^+/K^+ -ATPase (e). (B) The K^+ current-voltage relationships are schematically shown at normal and low $[K^+]_o$. At low $[K^+]_o$, the "negative resistance" due to closure of the K^+ inward rectifier causes a second stable membrane potential at which the muscle fibers are depolarized to -50 mV and paralyzed due to $Na_v1.4$ inactivation. Stable potentials with $I = 0$ (x axis) are in areas of the curve in which a depolarization can be compensated by a hyperpolarizing outward current (ascending curves at zero points).

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 Q_{ON} -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 $Ca_v1.1$ 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 $Na_v1.4$ repeats II (R1H, R2H/G/S/C) and III (R3Q, the outmost 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 I_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 $[\text{K}^+]_o$ 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 I_h 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 HCO_3^- to form H_2O and CO_2 in a reaction catalyzed by the myoplasmic carbonic anhydrase. The HCO_3^- could be supplied by the $\text{Na}^+/\text{HCO}_3^-$ 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 $[\text{K}^+]_o$ 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 $[\text{K}^+]_o$ 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^+ -mediated proton current cannot be balanced by the K^+ current as it strikingly is reduced at low $[\text{K}^+]_o$. 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 $[\text{K}^+]$, 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.

We would like to thank M. Fauler and B. Holzherr for fruitful discussions.

This work was supported by the German Research Foundation (DFG, JU470/1).

REFERENCES

- Campos, F.V., B. Chanda, B. Roux, and F. Bezanilla. 2007. Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. *Proc. Natl. Acad. Sci. USA*. 104:7904–7909.
- Cestele, S., T. Scheuer, M. Mantegazza, H. Rochat, and W.A. Catterall. 2001. Neutralization of gating charges in domain II of the sodium channel α subunit enhances voltage-sensor trapping by a β -scorpion toxin. *J. Gen. Physiol.* 118:291–302.
- Engel, A.G., E.H. Lambert, J.W. Rosevear, and W.N. Tauxe. 1965. Clinical and electromyographic studies in a patient with primary hypokalemic periodic paralysis. *Am. J. Med.* 38:626–640.
- Gadsby, D.C., and P.F. Cranefield. 1977. Two levels of resting potential in cardiac Purkinje fibers. *J. Gen. Physiol.* 70:725–746.
- Jurkat-Rott, K., F. Lehmann-Horn, A. Albaz, R. Heine, R.G. Gregg, K. Hogan, P.A. Powers, L.P.J.E. Vale-Santos, J. Weissenbach, and B. Fontaine. 1994. A calcium channel mutation causing hypokalemic periodic paralysis. *Hum. Mol. Genet.* 3:1415–1419.
- Jurkat-Rott, K., N. Mitrovic, C. Hang, A. Kouzmekine, P. Iaizzo, J. Herzog, H. Lerche, S. Nicole, J. Vale-Santos, D. Chauveau, et al. 2000. Voltage sensor sodium channel mutations cause hypokalemic periodic paralysis type 2 by enhanced inactivation and reduced current. *Proc. Natl. Acad. Sci. USA*. 97:9549–9554.
- Kuzmenkin, A., V. Muncan, K. Jurkat-Rott, C. Hang, H. Lerche, F. Lehmann-Horn, and N. Mitrovic. 2002. Enhanced inactivation and pH sensitivity of Na^+ channel mutations causing hypokalemic periodic paralysis type II. *Brain*. 125:835–843.
- Lehmann-Horn, F., and K. Jurkat-Rott. 1999. Voltage-gated ion channels and hereditary disease. *Physiol. Rev.* 79:1317–1371.

- Niall, J.F., and R.K. Pak Poy. 1966. Studies in familial hypokalaemic periodic paralysis. *Australas. Ann. Med.* 15:352–358.
- Ruff, R.L. 1999. Insulin acts in hypokalemic periodic paralysis by reducing inward rectifier K⁺ current. *Neurology.* 53:1556–1563.
- Sokolov, S., T. Scheuer, and W.A. Catterall. 2005. Ion permeation through a voltage-sensitive gating pore in brain sodium channels having voltage sensor mutations. *Neuron.* 47:183–189.
- Sokolov, S., T. Scheuer, and W.A. Catterall. 2007. Gating pore current in an inherited ion channelopathy. *Nature.* 446:76–78.
- Starace, D.M., and F. Bezanilla. 2001. Histidine scanning mutagenesis of basic residues of the S4 segment of the Shaker K⁺ channel. *J. Gen. Physiol.* 117:469–490.
- Starace, D.M., and F. Bezanilla. 2004. A proton pore in a potassium channel voltage sensor reveals a focused electric field. *Nature.* 427:548–553.
- Starace, D.M., E. Stefani, and F. Bezanilla. 1997. Voltage-dependent proton transport by the voltage sensor of the Shaker K⁺ channel. *Neuron.* 19:1319–1327.
- Struyk, A.F., and S.C. Cannon. 2007. A Na⁺ channel mutation linked to hypokalemic periodic paralysis exposes a proton-selective gating pore. *J. Gen. Physiol.* 130:11–20.
- Struyk, A.F., K.A. Scoggan, D.E. Bulman, and S.C. Cannon. 2000. The human skeletal muscle Na channel mutation R669H associated with hypokalemic periodic paralysis enhances slow inactivation. *J. Neurosci.* 20:8610–8617.
- Tombola, F., M.M. Pathak, and E.Y. Isacoff. 2005. Voltage-sensing arginines in a potassium channel permeate and occlude cation-selective pores. *Neuron.* 45:379–388.
- Tombola, F., M.M. Pathak, P. Gorostiza, and E.Y. Isacoff. 2007. The twisted ion-permeation pathway of a resting voltage-sensing domain. *Nature.* 445:546–549.
- Venkataraman, P., R.A. Lamb, and L.H. Pinto. 2005. Chemical rescue of histidine selectivity filter mutants of the M2 ion channel of influenza A virus. *J. Biol. Chem.* 280:21463–21472.
- Yang, N., A.L. George Jr., and R. Horn. 1996. Molecular basis of charge movement in voltage-gated sodium channels. *Neuron.* 16:113–122.