nature

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41586-018-0120-4

In the format provided by the authors and unedited.

Structural basis for gating pore current in periodic paralysis

Daohua Jiang[1,6], Tamer M. Gamal El-Din[1,6], Christopher Ing[2,3], Peilong Lu[1,4], Régis Pomès[2,3], Ning Zheng[1,5]* & William A. Catterall[1]*

[1]Department of Pharmacology, University of Washington, Seattle, WA, USA. [2]Molecular Medicine, Hospital for Sick Children Toronto, Toronto, Ontario, Canada. [3]Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada. [4]Institute for Protein Design, University of Washington, Seattle, WA, USA. [5]Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA. [6]These authors contributed equally: Daohua Jiang, Tamer M. Gamal El-Din. *e-mail: wcatt@uw.edu; nzheng@uw.edu

Supplementary Discussion

Nomenclature for Periodic Paralyses

The classic periodic paralyses include Hyperkalemic Periodic Paralysis (HyperPP), Hypokalemic Periodic Paralysis, and Paramyotonia Congenita[1]. Mutations that cause Normokalemic Periodic Paralysis were discovered much later[2], and there remains disagreement about the name for this syndrome because it does not describe the symptoms and differential diagnosis of all patients well. In HyperPP, HypoPP, and NormoPP, potassium sensitivity is a key diagnostic feature, and the names Potassium-sensitive HyperPP, Potassium-sensitive HypoPP, and Potassium-sensitive NormoPP have been suggested to be more accurate descriptions of the phenotypic characteristics[3,4]. Because this nomenclature remains an area of active discussion in clinical neurology, we have used the shorter traditional names for these syndromes here as they are the most familiar to a broad range of readers.

Functional Properties of Na_VAb/R2G, Na_VAb/R2S, and Na_VAb/R3G

Although virally infected High-Five cells expressed high levels of Na_VAb/R2G for structure determination, we were unable to record substantial sodium currents in these cells. Na_vAb/WT activates at very negative potentials and enters a slow inactivated state from which recovery is very slow[5]. It is likely that the voltage dependence of inactivation has been shifted to negative membrane potentials, inactivating all of the NaVAb/R2G channels, as we have found for other site-directed Na_VAb mutants. Na_VAb/R2S is the HypoPP mutant having the most similar in amino acid side chain to Na_VAb/R2G, so we chose it for functional characterization of a reconstituted R2 mutant gating pore. Consistent with the idea that mutations at R2 cause negatively shifted gating, Na_VAb/R2S activated and inactivated at a very negative membrane potential, and a holding potential of -200 mV, at the negative limit of cell stability, was required to study central pore currents reliably (Fig. 1a). The activation of central pore current was first observed at ~ -130 mV and reached saturation at ~ -60 mV (Fig. 1a). Steady state inactivation of the Na_VAb/R2S construct could not be studied because long times at -200 mV during the repeated pulse protocols required to measure steady state inactivation caused cell damage. Na_VAb/R3G activated at more positive membrane potentials, with initial inward current measured at ~ -50 mV and saturation at ~ +10 mV (Fig. 1d). We observed previously that substitutions at R3 cause a large positive shift in the voltage dependence of activation[6], consistent with these results. Because of this positive shift, we were able to use a holding potential of -160 mV and measure steady state inactivation of Na_VAb/R3G (Fig. 1d).

Amplitude of Gating Pore Current

In our previous studies of gating pore current in $Na_V1.2$/R2G, we found that the gating pore current was ~1% of the central pore current[7]. The large difference in amplitude of central pore current and gating pore current makes accurate estimation of their ratio difficult. In a few cases where we were able to measure central pore currents and gating pore currents of Na_VAb from the same cell, the ratio of the amplitude of I_{gp} measured at +50 mV compared

to I_{Na} of the central pore measured at 0 mV was 0.068 ± 0.010 (n=5). Since Na_vAb is a homotetrameric channel, there are four gating pores and one central pore; therefore, the ratio per VS is 0.017 or 1.7%. This value is very close to the estimate for mammalian sodium channels[7,8].

1 Venance, S. L., Cannon, S. C., Fialho, D., Fontaine, B., Hanna, M. G., Ptacek, L. J., Tristani-Firouzi, M., Tawil, R. & Griggs, R. C. The primary periodic paralyses: diagnosis, pathogenesis and treatment. *Brain* **129**, 8-17 (2006).

2 Vicart, S., Sternberg, D., Fournier, E., Ochsner, F., Laforet, P., Kuntzer, T., Eymard, B., Hainque, B. & Fontaine, B. New mutations of SCN4A cause a potassium-sensitive normokalemic periodic paralysis. *Neurology* **63**, 2120-2127 (2004).

3 Ptacek, L. J. & Fu, Y. H. Molecular biology of episodic movement disorders. *Adv Neurol* **89**, 453-458 (2002).

4 Renner, D. R. & Ptacek, L. J. Periodic paralyses and nondystrophic myotonias. *Adv Neurol* **88**, 235-252 (2002).

5 Gamal El-Din, T. M., Martinez, G. Q., Payandeh, J., Scheuer, T. & Catterall, W. A. A gating charge interaction required for late slow inactivation of the bacterial sodium channel NavAb. *J Gen Physiol* **142**, 181-190 (2013).

6 Gamal El-Din, T. M., Scheuer, T. & Catterall, W. A. Tracking S4 movement by gating pore currents in the bacterial sodium channel NaChBac. *Journal of General Physiology* **144**, 147-157 (2014).

7 Sokolov, S., Scheuer, T. & Catterall, W. A. Gating pore current in an inherited ion channelopathy. *Nature* **446**, 76-78 (2007).

8 Sigel, E. Properties of single sodium channels translated by Xenopus oocytes after injection with messenger ribonucleic acid. *J Physiol* **386**, 73-90 (1987).