Neurophysiological impact and modeling-independent elucidation of inactivation pathways in A-type K⁺ channels

JD Fineberg[1, 3], DM Ritter[2, 3] & M Covarrubias[1, 2, 3]

[1]Graduate Program in Molecular Physiology and Biophysics, [2]Graduate Program in Neuroscience, [3]Department of Neuroscience and Farber Institute for Neuroscience, Thomas Jefferson University, Philadelphia, PA

Abstract

A-type voltage-gated K⁺ (Kv) channels auto-regulate their function by undergoing fast inactivation. Independent of molecular mechanisms, this inactivation can proceed after channel opening (open-state inactivation, OSI) or from a closed state prior to opening (closed-state inactivation, CSI). We hypothesize that the specific neurophysiological roles of A-type Kv channels depend on whether they undergo OSI, CSI or both (CSI+OSI). To explore these possibilities, we introduced Markov kinetic schemes of the A-type Kv4 conductance into a computational model of the hippocampal CA1 neuron assuming either CSI or CSI+OSI and compared the properties of the somatic and dendritic action potentials (APs). Relative to the impact of CSI, the main differential effects of CSI+OSI are: 1) 15% less attenuation of back-propagating APs; 2) shorter latency to the first somatic spike; 3) exaggerated activity-dependent spike broadening and peak attenuation in somatic AP trains; and 4) the inter-spike interval of AP trains initially increases before it is shortened (CSI generates monotonic shortening). The outcome of these simulations thus motivated the development of a simple, modeling-independent method to conclusively elucidate the preferred pathways of inactivation in two distinct A-type Kv channels – Kv3.4 and Kv4.2 – expressed in heterologous cells and specific neurons. We applied two voltage-clamp pulse protocols – single- and double-pulse (modest conditioning step followed by a strong test step) – to obtain three pieces of critical information: development macroscopic single-pulse inactivation, the rate of double-pulse inactivation and the voltage-dependence of the time constant of macroscopic inactivation. Consistent with OSI, the rate of Kv3.4 inactivation precisely superimposes on the profile of the Kv3.4 current evoked by a single-pulse and the time constant vs. voltage relation decreases monotonically and levels off. By contrast, in ternary Kv4.2 channels, the rate of Kv4.2 inactivation is asynchronous, peaking earlier relative to the profile of the Kv4.2 single-pulse current and the time constant vs. voltage relation displays a 'J-shape' profile. Thus, Kv4.2 inactivation occurs uncoupled from channel opening, indicating CSI. Furthermore, removing KChIP1 from the Kv4 ternary complex or adding DPP10a to Kv4.2 channels produces a CSI+OSI phenotype. This procedure unambiguously establishes contrasting pathways of inactivation in neuronal A-type Kv channels, and provides a simple tool to correlate regulation of ionic conductance and neurophysiological activity.

Acknowledgements

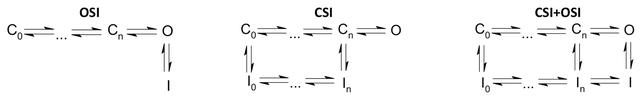
JDF was supported by the Department of Molecular Physiology and Biophysics of Jefferson Medical College (Dr. Marion J. Siegman, Professor and Chair). We thank the members of the Covarrubias lab and JDF thesis committee for helpful feedback. This work is supported by NIH grant R01 NS032337 (MC).

Summary & Conclusions

- We substituted Markov kinetic schemes of the A-type Kv4 conductance in a hippocampal CA1 neuron computational model assuming either CSI or CSI+OSI. When comparing the properties of the somatic and dendritic APs, relative to CSI only, the effects of CSI+OSI are:
  - 15% less attenuation of back-propagating APs;
  - shorter latency to the first somatic spike;
  - exaggerated activity-dependent spike broadening and peak attenuation in somatic AP trains;
  - the inter-spike interval of AP trains initially increases before it is shortened (CSI generates monotonic shortening).
- We implemented a simple method to conclusively determine the inactivation pathways of two Kv channels – Kv3.4 and Kv4.2 – expressed in heterologous cells and specific neurons. Using two voltage-clamp pulse protocols to observe development of single-pulse inactivation, the rate of double-pulse inactivation and the voltage-dependence of the time constant of inactivation we can conclude:
  - Heterologous and native Kv3.4 channels demonstrate an exact superimposition of the development of single-pulse inactivation and the rate of double-pulse inactivation and therefore undergo *preferential* OSI.
  - The development of single-pulse inactivation and rate of double-pulse inactivation in heterologous and native Kv4.2 channel complexes are asynchronous. Combined with the 'J' -shape voltage dependence of inactivation time constants, we can conclude these channels undergo *preferential* CSI.
  - By removing KChIP1 from the Kv4 ternary complex or by replacing DPP6 with DPP10a abolishes the 'J'-shape while preserving the lack of of superimposition of the development of single-pulse inactivation and the rate of double-pulse inactivation. We can conclude that these subunit manipulations produce a CSI+OSI phenotype.
- This procedure unambiguously establishes contrasting pathways of inactivation in neuronal A-type Kv channels, and provides a simple tool to investigate regulation of ionic conductance and neurophysiological activity.

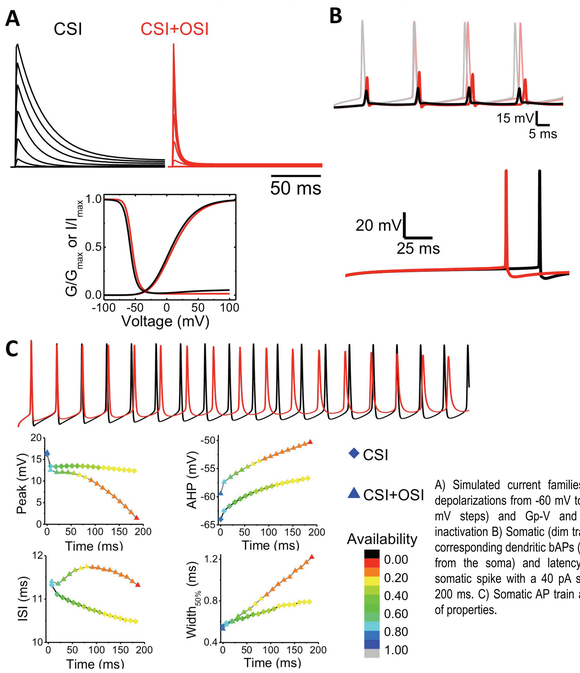
Introduction

Inactivation is an intrinsic autoregulatory mechanism of A-type voltage-gated K⁺ channels (Bähring and Covarrubias, 2011), and may occur from the closed (activated) and/or open states (CSI or OSI, respectively).



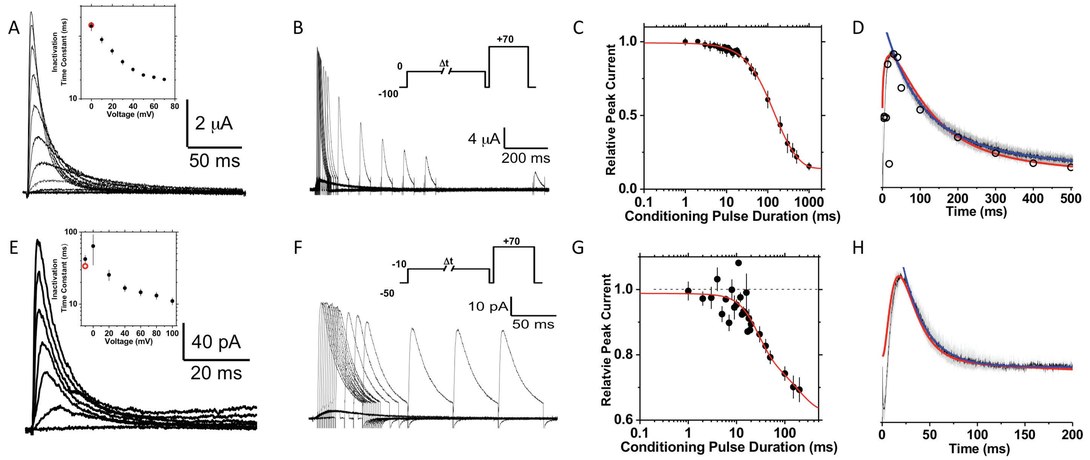
- The first objective of this work was to explore whether the pathways of inactivation of neuronal A-type K⁺ channels may influence the physiological activity of a neuron. Therefore, we exploited a previously reported computational model of the hippocampal CA1 neuron (Migliore et al., 1999), replacing the conventional Hodgkin and Huxley model of the A-type K⁺ conductance with Markov kinetic schemes that specifically assume either preferential CSI or CSI+OSI.
- The second objective was to establish a simple electrophysiological procedure that in an unambiguous and modeling-independent manner may determine the pathways of inactivation in native and heterologously expressed Kv channels. Furthermore, this procedure may help investigate how subunit composition influences the pathways of inactivation. Inspired by a study of sodium channel inactivation (Bean, 1981), we used two simple voltage-clamp pulse protocols to obtain three pieces of critical information – development macroscopic single-pulse inactivation, the rate of double-pulse inactivation and the voltage-dependence of the time constant of macroscopic inactivation – to determine which inactivation pathways are used, and if one is used preferentially, in six different channels/complexes.

Figure 1. Neurophysiological impact of Kv4 channel inactivation pathways in a computational model



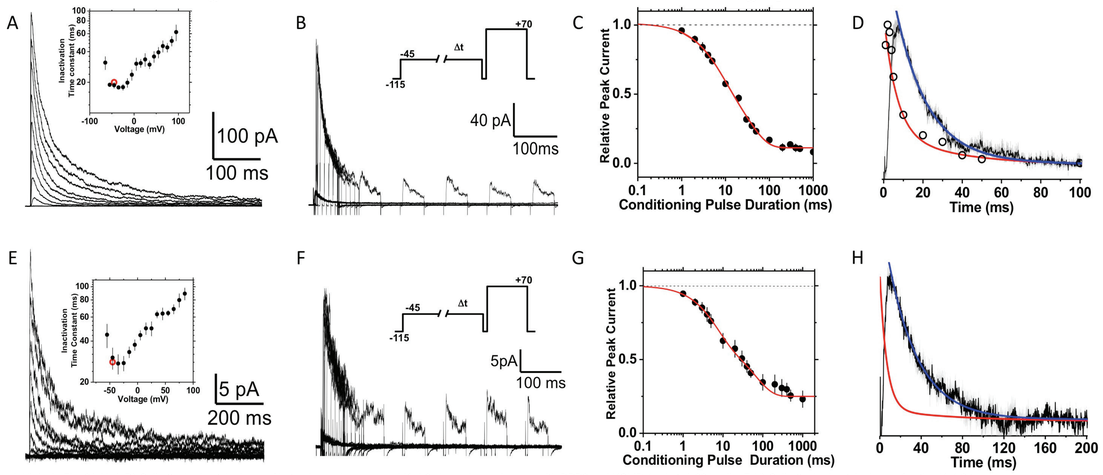
A) Simulated current families evoked by depolarizations from -60 mV to +80 mV (20 mV steps) and Gp-V and steady-state inactivation B) Somatic (dim traces) and the corresponding dendritic bAPs (solid, 400 µm from the soma) and latency to the first somatic spike with a 40 pA stimulation for 200 ms. C) Somatic AP train and summary of properties.

Figure 2. Recombinant and native Kv3.4 channels undergo preferential OSI



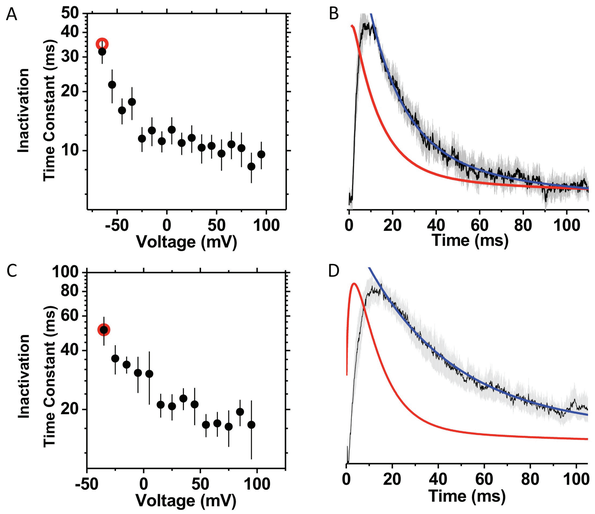
A-D) Whole-oocyte voltage clamp of recombinant Kv3.4 channels. A) Family of outward currents evoked by a series of voltage steps. Time constants or weighted time constants were determined from the best-fit exponential or sum of two exponentials that describes the decay phase of the currents, respectively, and plotted against voltage (inset). For comparison, the red symbol in indicates the time constant determined from the double-pulse protocol. B) Outward currents evoked by a double-pulse protocol (inset), where the conditioning pulse activated 10-20% of the peak conductance. C) Kinetics of double-pulse inactivation. The normalized peak current evoked by the test pulse of the protocol in panel B plotted against the duration of the conditioning pulse (filled circles). The solid red line represents the empirical best-fit sum of exponential terms. D) Overlay of the average outward current (mean ± SEM, black and gray, respectively) evoked by a single pulse and the rate of inactivation (red) at the voltage indicated in panel B, inset. The rate of inactivation is the scaled negative first derivative of the red solid line in panel E. The blue solid line is the exponential function that best describes the decay phase of the current trace (black). The scaled negative first derivative of the observed time course of double-pulse inactivation is also plotted (open black circles) for comparison. E-H) As in A-D for cell-attached patch clamp of native Kv3.4 channels in DRG neurons.

Figure 3. Recombinant and native Kv4.2 channels undergo preferential CSI



A-H) As in Fig. 2A-H. A-D) Cell-attached patch clamp of heterologously expressed Kv4.2 channel complexes – with DPP6 and KChIP-1 – in tsA 201 cells. E-H) Cell-attached patch clamp of native Kv4.2 ternary channels in cerebellar granule neurons.

Figure 4. Subunit composition of Kv4.2 channel complexes determines the pathway of inactivation



A-D) Cell-attached patch clamp of heterologously expressed Kv4.2 channel complexes – without KChIP-1 (A&B) or with DPP10a substituted for DPP6 (C&D) – in tsA 201 cells. A&C) As in Fig. 3A, inset. B&D) As in Fig. 3D.

References

Amarillo Y, et al. (2008). *J Physiol* **586.8**, 2093–2106; Bähring R & Covarrubias M (2011). *J Physiol* **589.3**, 461-479; Bean BP (1981). *Biophys. J* **35**, 595-614; Fineberg JD, et al. (2012). *J Gen Physiol* (in press); Kaulin, et al. (2008). *Biophys J*, **94**, 1241-51; Kaulin, et al. (2009). *J Neurosci*, **29**,3242-3251; Migliore M, et al. (1999). *J Comput Neurosci*, **7**, 5-15; Ritter DM, et al. (2012). *J Physiol*, **590**, 145-161.

Experimental Methods

Simulations were implemented in the NEURON simulation environment (v7.1) using the Migliore *et al.* (1999) model of a hippocampal neuron. To obtain Markov CSI and CSI+OSI models, IChMASCOT (www.jadesantiago.com/Electrophysiology/IChMASCOT/) was used to determine the best global fit to theoretical currents generated by the Hodgkin-Huxley model of distal K_A channels. The CSI and CSI+OSI models were then used to replace the Hodgkin-Huxley K_A currents and simulations conducted. All files used in simulations are available on the ModelDB website under the accession number 145672. The modeling package IonChannelLab (Jose De Santiago-Castillo; http://jadesantiago.com) was used to simulate the K⁺ currents predicted by specific kinetic schemes.

For heterologous expression, tsA 201 cells were co-transfected with plasmids for each of the Kv4.2 ternary channel complex subunit cDNAs as described by Amarillo, *et al.* (2008), or Kv3.4 mRNA was microinjected into defolliculated *X. laevis* oocytes (50 ng/cell) with a Nanoject microinjector. Patch-clamp recordings were performed using standard cell-attached configuration as described by Kaulin, *et al.* (2009). Two-electrode voltage-clamping experiments of Kv3.4 channels were performed as previously described by Kaulin, *et al.* (2008). Acutely dissociated CGNs and DRG neurons were obtained from 7-8 day old and adult Sprague-Dawley rats, respectively, as described by Kaulin, *et al.* (2009) and Ritter, *et al.* (2012), respectively. Recording of native channels from CGNs was performed using standard cell-attached configuration, similarly to the tsA 201 recording, as described by Fineberg, *et al.* (2012). Recording of native channels from DRG neurons was performed using standard cell-attached configuration as previously described by Ritter, *et al.* (2012). ClampFit 10.0 and OriginPro 8.0 were used to perform data analysis and empirical curve fitting. All data was obtained at room temperature (~23º C) and acquired with the Clampex software. All data is shown as mean ± SEM, n = 5-10 patches.