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

Abstract

A-type voltage-dependent 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, CSI) or from a closed state prior to opening (clamping inactivation, CSI). We hypothesize that the specific neurophysiological roles of A-type Kv channels depend on whether CSI, OSI or both (CSI+OSI) are involved. To explore these possibilities, we introduced Marston kinetic schemes of the A-type Kv4 conductance into a computational model of the hippocampal CA1 neuron assuming either CSI, OSI or CSI+OSI. We predicted the effects of CSI on the development of single-channel inactivation and the voltage-dependence of the time constant of inactivation. Consistent with CSI, the results of the simulation process superimpose on the profile of Peak VI current evoked by a single and the single time constant vs. voltage relationship monotonically and levels off by contrast; ternary Kv4.2 channels, the rate of Kv4.2 inactivation is asymptotic, peaking earlier relative to the profile of the Kv4.2 single pulse-current and the time constant vs. voltage relationship 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 inactivation of native neuronal A-type Kv channels, and provides a simple tool to ascertain regulations of ionic conductance and neurophysiological activity.

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Introduction

Inactivation is an intrinsic autoregulatory mechanism of A-type voltage-gated K⁺ channels (Böhmig 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 native A-type Kv channels may influence the neurophysiological activity. In such a manner, we explored previously reported Hodgkin and Huxley model of the A-type Kv4 channel-inactivation with Marston kinetic schemes that specifically assume either preferred CSI or 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 ion channel composition influences the pathways of inactivation. Inspired by a study of sodium channel inactivation (Esaias et al., 1985), 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 there is a preferred CSI vs. OSI.

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

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

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

Summary & Conclusions

• We substituted Marston kinetic schemes of the A-type Kv4 conductance in a hippocampal CA1 neuron modeling computational model assuming either CSI or CSI+OSI. When comparing the properties of the somatic and dendritic APs, relative to CSI, the effects of CSI are:
  - 15% less attenuation of backpropagating 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 monadotic inactivation).

• 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 conclude that by removing KChIP1 from the Kv4 ternary complex or by replacing DPP6 with DPP10a abolishes the 'J'-shape while preserving the lack of superimposition of the development of single-pulse inactivation and the rate of double-pulse inactivation. We can conclude that the subunit manipulations produce CSI+OSI phenotype.

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

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Abstract

Electrophysiological experiments of Kv4.2 channels were performed in hippocampal CA1 neurons assuming either CSI, OSI or CSI+OSI. The conclusions were based on the effects of CSI on the development of macroscopic single-pulse inactivation, the rate of double-pulse inactivation and the voltage-dependence of the time constant of inactivation. Consistent with CSI, the results of the simulation process superimposed on the profile of Peak VI current evoked by a single pulse and the time constant vs. voltage relationship displayed a ‘J-shape’ profile. Thus, Kv4.2 inactivation occurred uncoupled from channel opening, indicating CSI. Furthermore, removing KChIP1 from the Kv4 ternary complex or adding DPP10a to Kv4.2 channels produced a CSI+OSI phenotype. This procedure unambiguously establishes contrasting inactivation of native neuronal A-type Kv channels, and provides a simple tool to investigate regulation of ionic conductance and neurophysiological activity.

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