External Pore Collapse as an Inactivation Mechanism for Kv4.3 K⁺ Channels

M. Eghbal1, R. Olcese1, M.M. Zarej1, L. Toro1,2,3, E. Stefani1,2,4
1Department of Anesthesiology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095-7155, USA
2Department of Physiology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095-7155, USA
3Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095-7155, USA
4Brain Research Institute, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095-7115, USA

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Abstract. Kv4 channels are thought to lack a C-type inactivation mechanism (collapse of the external pore) and to inactivate as a result of a concerted action of cytoplasmic regions of the channel. To investigate whether Kv4 channels have outer pore conformational changes during the inactivation process, the inactivation properties of Kv4.3 were characterized in 0 mM and in 2 mM external K⁺ in whole-cell voltage-clamp experiments. Removal of external K⁺ increased the inactivation rates and favored cumulative inactivation by repetitive stimulation. The reduction in current amplitude during repetitive stimulation and the faster inactivation rates in 0 mM external K⁺ were not due to changes in the voltage dependence of channel opening or to internal K⁺ depletion. The extent of the collapse of the K⁺ conductance upon removal of external K⁺ was more pronounced in NMG⁺-than in Na⁺-containing solutions. The reduction in the current amplitude during cumulative inactivation by repetitive stimulation is not associated with kinetic changes, suggesting that it is due to a diminished number of functional channels with unchanged gating properties. These observations meet the criteria for a typical C-type inactivation, as removal of external K⁺ destabilizes the conducting state, leading to the collapse of the pore. A tentative model is presented, in which K⁺ bound to high-affinity K⁺-binding sites in the selectivity filter destabilizes an outer neighboring K⁺ modulatory site that is saturated at ~2 mM external K⁺. We conclude that Kv4 channels have a C-type inactivation mechanism and that previously reported alterations in the inactivation rates after N- and C-termini mutagenesis may arise from secondary changes in the electrostatic interactions between K⁺-binding sites in the selectivity filter and the neighboring K⁺-modulatory site, that would result in changes in its K⁺ occupancy.

Key words: K channel — Inactivation — Kv4 — Kv4.3 — C-inactivation — Ito — Channel inactivation

Introduction

K⁺ channel activity is modulated by external and internal K⁺ ions. This general property of K⁺ channels was early observed by Almers and Armstrong (1980) in the squid giant axon K⁺ channels. K⁺ channels lost their function when K⁺ was eliminated from both sides of the membrane. It was concluded that K⁺ channels were normally occupied by K⁺ and/or other small monovalent cations and that they become nonfunctional after the removal of internal and external K⁺ ions (Almers & Armstrong, 1980). These initial observations were the basis for understanding the mechanism(s) of action of K⁺ ions and other cations in several classes of K⁺ channels (Pardo et al., 1992; Lopez-Barneo et al., 1993; Yellen et al., 1994; Baukrowitz & Yellen, 1995; Liu, Jurman & Yellen, 1996). Since the effect of removing external K⁺ depends on the K⁺ affinity of the K⁺ channel site, some channels can function properly in “zero” mm external K⁺ (contaminant K⁺ ~10 µM) due to their high affinity to K⁺. This is the case for the large-conductance, voltage-dependent and Ca²⁺-activated K⁺ (MaxiK, BK) channels. In this channel, reducing further the external K⁺ concentration with a K⁺ chelator ((+)-18-Crown-6-tetracarboxylic

Correspondence to: E. Stefani; email: estefani@ucla.edu
acid), causes them to enter a long-lasting non-conductive state (Vergara et al., 1999).

Shaker K⁺ channels inactivate through two distinct molecular mechanisms, N- and C-type inactivation. N-type inactivation involves the N-terminal domain with the already classical “ball and chain” mechanism, while C-type inactivation is due to structural modifications of the external mouth of the pore (Armstrong & Bezanilla, 1977; Zagotta et al., 1990; Hoshi et al., 1990; Hoshi, Zagotta & Aldrich, 1991; Yellen et al., 1994; Panyi, Zheng & Deutsch, 1995; Liu et al., 1996; Basso et al., 1998; Loots & Isacoff, 1998; Zhou et al., 2001). The following observations in Shaker type (Kv1) K⁺ channels suggest that external K⁺ and small cations modulate C-type inactivation and channel recovery from the inactivated state: 1. External tetraethylammonium (TEA⁺) reduces C-type inactivation (Grissmer & Cahalan, 1989a; Choi, Aldrich & Yellen, 1991). 2. C-type inactivation rate depends on external K⁺; decreasing K⁺ accelerates inactivation, suggesting that K⁺ ions occupying the outer region of the pore prevent its collapse and compete with the inactivation process. The relative potency of external cations in accelerating C-type inactivation is NMG⁺ > Na⁺ ~ Cs⁺ ~, NH₄⁺ > K⁺ ~ Rb⁺ (Pardo et al., 1992; Lopez-Barneo et al., 1993). 3. Internal K⁺ channel blockers increase C-type inactivation rate, as they prevent outward K⁺ flux (Choi et al., 1991; Baurkowitz & Yellen, 1995; Baurkowitz & Yellen, 1996). 4. Increasing external K⁺ speeds recovery from C-type inactivation via a voltage-dependent binding of K⁺ to an extracellular site (Levy & Deutsch, 1996). Thus, as a general mechanism, the inactivation time constant of C-type inactivation depends on the occupancy by K⁺ of an external modulatory site. In other words, K⁺ channels need external K⁺ to function properly, and removal of external K⁺ favors C-type inactivation by promoting a constriction or partial collapse of the external region of the pore (Yellen, 1998).

More recently, it has been demonstrated that the occupancy of the external modulatory site does not only depend on the external K⁺ concentration and/or K⁺ flux, but also on the K⁺ affinity of a site within the channel pore. A decrease in the affinity of a site within the channel pore would reduce electrostatic interactions between the K⁺ ions in the pore and in the external modulatory site and, as a consequence, the occupancy of the modulatory site would increase, slowing down C-type inactivation. Conversely, an increase in K⁺ affinity of the site within the pore will have opposite affects, destabilizing the occupancy of the modulatory site and favoring C-type inactivation (Ogielska & Aldrich, 1999).

The role of external K⁺ has been extensively studied in Shaker-type K⁺ channels; however, a detailed study on the effect of removing external K⁺ on Kv4 channels is lacking. Kv4 channels are one of the molecular components of the outward K⁺ currents that shape the early repolarization phase of the action potential (commonly referred to as “A-type” or “Ito” current, (Serodio & Rudy, 1998; Isbrandt et al., 2000). The rate of inactivation is one of the important intrinsic properties of Kv4 channels controlling the action potential shape. Kv4 inactivation can be described with two or three components of decay and the predominant mechanism seems to be the population of inactivation from pre-open closed states (Jerng & Covarrubias, 1997; Jerng, Shahidullah & Covarrubias, 1999; Bähring et al., 2001; Beck & Covarrubias, 2001). In Kv4 channels, an increase of the external K⁺ concentration produces an acceleration of their inactivation process (Jerng & Covarrubias, 1997; Bähring et al., 2001; Eghbali et al., 2001). This is in contrast with C-type inactivation in Shaker K⁺ channels, in which raising external K⁺ causes a reduction in the rate of inactivation, likely due to an increased occupancy of an external modulatory K⁺ site (Lopez-Barneo et al., 1993). In addition, the recovery from inactivation in Kv4 channels is slower in high external K⁺, which is also opposite to what is found in Kv1 channels where the recovery of C-type inactivation is faster in high external K⁺ (Levy & Deutsch, 1996; Jerng & Covarrubias, 1997).

In Kv4 channels, molecular maneuvers to examine its inactivation mechanism show that an N-terminus deletion removes or greatly slows down the fast component of inactivation (τ = 16 ms) without affecting a slower inactivating component (τ ~ 1000 ms) associated with closed state inactivation. In addition, mutations in the inner vestibule of the Kv4 pore drastically slowed inactivation rates and reduced Kv4 4-aminopyridine blockade. Based on all the above results in Kv4 channels, it has been proposed that Kv4 channels lack typical N- and C-type inactivation as described for Shaker K⁺ channels, but possess an inactivation mechanism that may involve voltage-associated conformational changes of the internal vestibule in a concerted action involving N- and C-termini (Jerng & Covarrubias, 1997; Jerng et al., 1999; Bähring et al., 2001). Nevertheless, removing external K⁺ using Na⁺ or NMG⁺ as the main cations, a landmark maneuver to investigate C-type inactivation, has not been addressed for Kv4 channels (Yellen, 1998). In this work we now characterize the inactivation process of Kv4.3 channels upon removal of external K⁺. We found that external K⁺ removal increased the inactivation rate and facilitated cumulative inactivation by repetitive stimulation. This effect was more pronounced when external Na⁺ was replaced by NMG⁺. The faster inactivation rate and current reduction during cumulative inactivation by repetitive stimulation occurred without changes of the voltage dependency of channel opening or of the internal K⁺ concentration.