Abstract

Voltage-gated calcium channels (VGCCs) control Ca^{2+} entry in excitable cells. The Ca^{2+} influx through VGCCs is initiated by the membrane depolarization and terminated by the channel inactivation, either calcium-dependent (CDI) or voltage-dependent (VDI). VGCCs consist of a pore-forming α_1-subunit defining the major biophysical properties, and auxiliary $\alpha_2\delta$-, β-, and sometimes γ-subunits. VGCCs with α_1-subunit encoded by the $Ca_V1.3$ gene belong to the family of L-type VGCCs.

$Ca_V1.3$ channels are expressed in central neurons, heart, endocrine cells, auditory hair cells, and photoreceptors (Catterall et al., 2005). Of note, the activation range of $Ca_V1.3$ channels coincides with the operating voltage range of sinoatrial node and cochlear inner hair cells, where these channels play a unique role in pacemaking (diastolic depolarization) and synaptic sound encoding (neurotransmitter release), respectively (Platzer et al., 2000). Loss of $Ca_V1.3$ function in humans and animal models leads to bradycardia (Matthes et al., 2004) and congenital deafness (Baig et al., 2011; Platzer et al., 2000). Furthermore, $Ca_V1.3$ channels mediate autonomous firing of dopamine neurons in the substatia nigra pars compacta (SNc) and may be potentially involved in the pathogenesis of Parkinson's and Alzheimer's diseases (Kim and Rhim, 2011; Surmeier et al., 2010). To limit potentially toxic Ca^{2+} entry into the cell, L-type Ca^{2+} channels inactivate via rapid calcium-dependent inactivation (CDI) or slower voltage-dependent inactivation (VDI). In CDI, elevated intracellular Ca^{2+} concentration is sensed by a small calcium-binding protein, calmodulin (CaM), which is pre-bound to conserved proximal C-terminal segments of the Ca_V channel pore (Halling et al., 2005).

The C-terminus of $Ca_V1.3$ channels is subject to alternative splicing. In the current study, two naturally occurring isoforms, $Ca_V1.3_{42}$ and $Ca_V1.3_{42A}$ were compared (Bock et al., 2011; Singh et al., 2008). The full-length $Ca_V1.3_{42}$ isoform contains a C-terminal modulator (CTM) domain, which is truncated in the $Ca_V1.3_{42A}$ isoform. CTM was shown to shift channel activation to more positive voltages and attenuate apparent CDI (Bock et al., 2011; Singh et al., 2008). It was proposed that CTM interferes with the binding of calmodulin (CaM) to an IQ domain in the proximal C-terminus of $Ca_V1.3$ channels and that the lack of CaM binding suppresses the channel activity and prevents CDI (Adams et al., 2014).

To study mechanisms of CTM-mediated inhibition of $Ca_V1.3$ channels in detail, we conducted single-channel measurements. With Ba^{2+} as a charge carrier, we found that $Ca_V1.3_{42A}$ channels displayed a high activity, which would correspond to a state with apo-CaM bound (Adams et al., 2014). Accordingly, $Ca_V1.3_{42}$ channels containing CTM showed a low channel open probability. However, in some experiments with single $Ca_V1.3_{42}$ channels, we observed

switches between high and low channel activity, likely reflecting binding and unbinding of CaM on a sub-minute time-scale.

With Ca^{2+} as a charge carrier at the single-channel level, CDI is manifested by the shortening of the open times and reducing the frequency of re-openings (Imredy and Yue, 1994). Josephson et al. showed a correlation between the accumulating amount of calcium ions passing through the channel and the subsequent shortening of mean open time (Josephson et al., 2010). We used coefficients of such correlations to quantify the CDI sensitivity. CDI sensitivity appeared to be larger for $Ca_V1.3_{42A}$ than for $Ca_V1.3_{42}$ isoform. Furthermore, we observed that Ca^{2+} influx from a few single-channel openings was sufficient to cause CDI of the channel. Though, CTM of $Ca_V1.3_{42}$ channels inhibited CDI compared with $Ca_V1.3_{42A}$ channels, CDI was not completely abolished. For most of the $Ca_V1.3_{42}$ channels recorded, we found switches between high and low CDI levels. Interestingly, the augmentation of the channel activity by the calcium channel agonist (S)-BayK 8644 led to pronounced CDI for both isoforms. To summarize, single-channel recordings allowed us to resolve equilibrium transitions between high and low channel activity and CDI of $Ca_V1.3$ channels affected by CTM and CaM.