



Cav3 Calcium Channel Interactions with Potassium Channels

Ray W. Turner

Abstract

The influence of potassium channels on cell activity is markedly enhanced by exhibiting the low voltage for activation, fast activation, and fast inactivation that characterize an A-type channel profile. Previous work had identified only a subset of members of the voltage-gated K_v1 , K_v3 , and K_v4 families in which alpha subunits are able to exhibit A-type biophysical properties. Recent work has shown that Cav3 channels closely associate with different potassium channels to impart their biophysical and kinetic properties and effectively create three new forms of A-type potassium channel. Ca_v3 -K channel interactions identified to date include Ca_v3 - K_v4 , Ca_v3 -IK, and Ca_v3 -BK that enable novel aspects of synaptic processing and spike discharge in cerebellar granule, stellate, Purkinje, and medial vestibular neurons. This chapter will highlight data obtained in each of these cell classes of how Ca_v3 associations with different potassium channels extend the number of channels capable of exhibiting an A-type phenotype to control cell activity.

Keywords

$Ca_v3.x$ · T-type · K_v4 · KCa3.1 · KCa1.1 · A-type

Introduction

Potassium channels control an enormous array of functions in setting the intrinsic excitability of neurons and shaping the pattern and frequency of spike output that defines the neural code used for signal processing. The most common biophysical phenotype of these channels is that of a relatively high voltage-activated (HVA) and non-inactivating outward current first recognized as a “delayed rectifier” in axon membranes. A distinct set of potassium channel properties came to be recognized with reports of a fast activating and fast inactivating *Drosophila* Shaker channel and its mammalian homolog, $K_v1.4$. This transient “A-type” outward current further exhibited properties of low voltage-activation (LVA) that allowed it to function well within the resting membrane potential range and provide unique contributions to spike frequency and timing following membrane hyperpolarizations. The importance of expressing A-type potassium channels is evident in an almost ubiquitous expression of this form of outward current in central neurons. Additional classes of voltage-gated potassium channels in which the alpha subunit

R. W. Turner (✉)
Hotchkiss Brain Institute, Alberta Children’s Hospital
Research Institute, Department of Cell Biology and
Anatomy, Cumming School of Medicine, University
of Calgary, Calgary, AB, Canada
e-mail: rwtturner@ucalgary.ca

alone exhibits A-type properties were eventually recognized, but it includes a relatively limited subset of members of the K_v1 ($K_v1.4$), K_v3 ($K_v3.3$, $K_v3.4$) and K_v4 ($K_v4.1$, 4.2 , 4.3) families.

By comparison, the fast activation and inactivation of voltage-gated A-type channels were rarely attributed to calcium-gated potassium channels. The properties of calcium-gated potassium channels had been shown amenable to change by establishing a close association and functional coupling to voltage-gated calcium channels. One illustration of this was in the functional coupling of $KCa1.1$ (big conductance, BK) potassium channels to high voltage-activated (HVA) but slow inactivating calcium channels (Robitaille et al., 1993; Poolos & Johnston, 1999; Grunnet & Kaufmann, 2004; Berkefeld et al., 2006, 2010; Loane et al., 2007; Müller et al., 2007; Berkefeld & Fakler, 2008; Marcantoni et al., 2010; Vandael et al., 2010). Other work established functional coupling at even the nanometer level between HVA calcium channels and both SK ($KCa2.x$) and IK ($KCa3.1$) potassium channels (Marrion & Tavalin, 1998; Lima & Marrion, 2007; Sahu et al., 2017, 2019; Sahu & Turner, 2021). A similar functional coupling between the LVA class of Ca_v3 calcium channels and potassium channels also came to be recognized (Smith et al., 2002; Wolfart & Roeper, 2002; Cueni et al., 2008; Gittis et al., 2010).

One region that received extensive work on the role of Ca_v3 channels in controlling cell output is the cerebellum. The cerebellum is comprised of highly organized arrays of four main classes of interneurons (granule, Golgi, stellate, basket) and 3 classes of output cells in cortical Purkinje cells and the deep cerebellar nuclear and vestibular neurons that receive Purkinje cell input. The expression pattern of Ca_v3 channel isoforms in cerebellar neurons has been reported in numerous studies (Bossu et al., 1989; Mougnot et al., 1997; Talley et al., 1999; Isope & Murphy, 2005; McKay et al., 2006; Molineux et al., 2006; Hildebrand et al., 2009; Anderson et al., 2010a, 2013; Engbers et al., 2012; Isope et al., 2012; Heath et al., 2014; Aguado et al., 2016; Ly et al., 2016). Through this work, it became apparent

that all Ca_v3 channel isoforms are widely expressed across the seven cell types in the cerebellum. The extent of Ca_v3 channel expression was surprising as it did not hold a clear relationship to the ability of different cells to exhibit a rebound discharge thought characteristic of cells that express T-type channels (Molineux et al., 2006). A series of studies investigating how Ca_v3 and potassium channels might work in tandem to control spike output revealed a rich repertoire of Ca_v3 influence over spike discharge through coupling with potassium channels. This chapter will focus on Ca_v3 channel interactions that have been found with either voltage- or calcium-gated potassium channels in cerebellar stellate and granule cells, Purkinje cells, and medial vestibular cells that receive input from Purkinje cells.

Ca_v3 channel isoforms have now been recognized to form a close association at the molecular level with three different potassium channel classes (Fig. 1). The end result of the Ca_v3 channel link to these different channels is unique in effectively creating three new classes of LVA, fast activating and fast inactivating forms of potassium channel that take on specific roles in different cerebellar cell types.

Ca_v3 - K_v4 Complex

The presence of immunolabel for Ca_v3 isoforms in cerebellar stellate and granule cells of lobule 9 despite a lack of rebound burst response following membrane hyperpolarizations encouraged further study of the role of Ca_v3 channels in these cells (Molineux et al., 2005; Heath et al., 2014; Rizwan et al., 2016). Recordings in stellate and granule cells confirmed the expression of both T-type calcium and K_v4 -mediated A-type potassium current. Surprisingly, it was found that the $K_v4.2$ A-type current was sensitive to T-type channel blockers. Specifically, bath perfusion of mibefradil (0.5 μ M) or low calcium medium (0.1 mM) selectively shifted the half inactivation voltage (V_h) of K_v4 current to more negative potentials (“left-shifted V_h ”) (Fig. 2a) (Molineux et al., 2005; Anderson et al., 2010a, 2013). No effect was detected on the half activation voltage

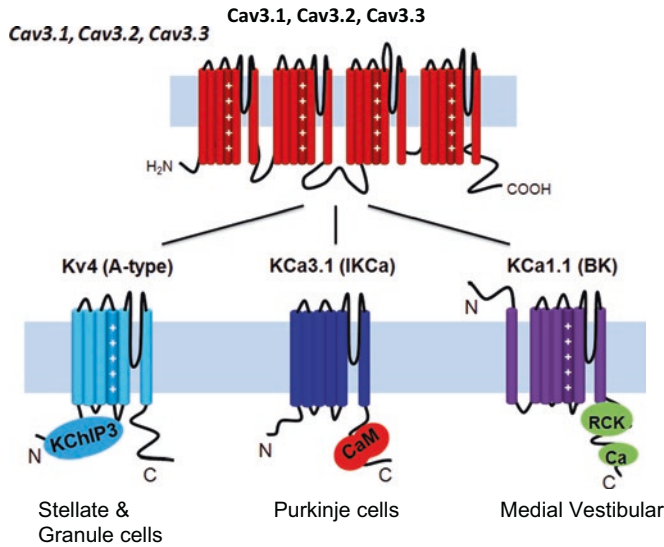


Fig. 1 T-type calcium channels couple at the molecular level to distinct potassium channels to invoke A-type current properties. Cartoon representation of $Ca_v3.x$ channel isoforms and three potassium channels they link to in cerebellar neurons to modulate spike output: voltage-gated $K_v4.x$ A-type channels in stellate and granule cell interneurons, calcium-gated channels $KCa3.1$ (IK) in Purkinje

cells, and $KCa1.1$ (BK) channels in medial vestibular neurons. Abbreviations: *N* N-terminus, *C* C-terminus, *KChIP3* potassium channel interacting protein 3, *CaM* calmodulin, *RCK* the regulator of conductance of potassium. (Figure is modified from Turner and Zamponi (2014))

(Va) of K_v4 current (Fig. 2a), current density, kinetics, or rate of recovery from inactivation. The net effect of reducing Ca_v3 calcium conductance was to decrease K_v4 current amplitude over a range of holding membrane potentials of ~ -50 to -90 mV (Fig. 2a). By focally ejecting low calcium medium (0.1 mM) the actions of Ca_v3 calcium influx on K_v4 Vh were further shown to be rapidly induced and fully reversible (Anderson et al., 2013). The application of a dynamic clamp of both Ca_v3 and K_v4 current in granule cells allowed for a reduction of K_v4 current amplitude over the appropriate voltage range in otherwise untreated cells (Heath et al., 2014). The importance of the Ca_v3 -mediated control over K_v4 Vh was immediately evident in a substantial increase in spike firing rate gain (Fig. 2b, c).

These studies were the first to report a calcium-dependent change in K_v4 channel properties, a channel that had only been recognized as exhibiting voltage-dependent properties. This raised interest in the potential role of a class of calcium-sensing KChIP proteins that had been found to

associate with K_v4 channels, but which had no identified role. Stellate cells were found to exhibit immunolabel for the KChIP3 isoform as well as a specific isoform of a second K_v4 accessory protein dipeptidyl peptidase 10c (DPP10c). Recordings of K_v4 current were used to assess the interaction between these subunits in stellate cells or when coexpressed in human embryonic kidney (HEK) cells. These and other tests revealed co-immunoprecipitation between $Ca_v3.1$ or $Ca_v3.3$ with K_v4 channel isoforms and KChIP3. Moreover, all of the effects of reducing Ca_v3 channel conductance on K_v4 Vh could be recapitulated by coexpressing these proteins in HEK cells together with DPP10c (Anderson et al., 2010a). Infusing through the electrode a Pan-KChIP or KChIP3 antibody, but not an antibody against KChIP1 reproduced the left-shift in K_v4 Vh by ~ -10 mV. These recordings thus established an absolute dependence of the left-shift in K_v4 Vh on coexpression of $Ca_v3.3$ and KChIP3, with similar but slightly less pronounced effects by $Ca_v3.1$ (Anderson et al., 2010a, b). It was

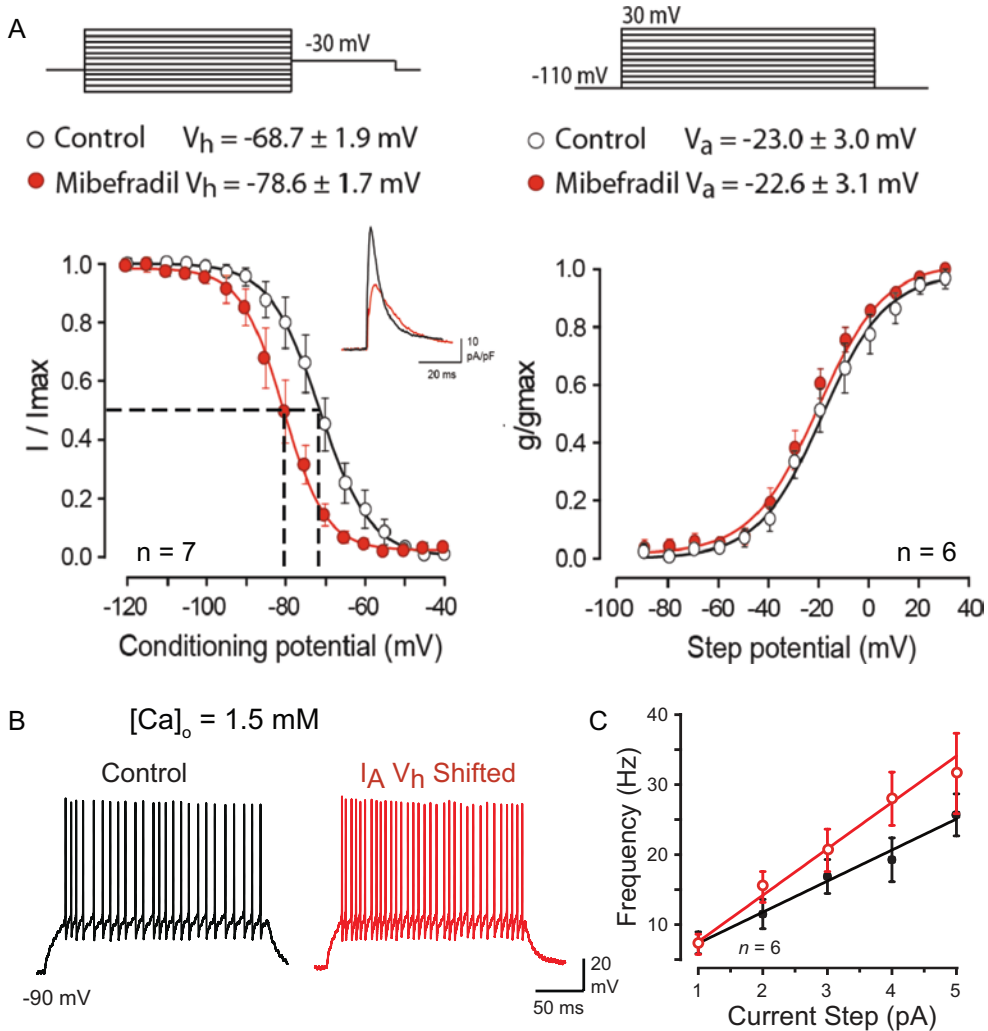


Fig. 2 A Ca_v3 - K_v4 complex shifts K_v4 availability to reduce spike output. (a) Mean plots of the voltage of inactivation and activation of K_v4 A-type potassium current in whole-cell recordings from cerebellar stellate cells in 1.5 mM $[Ca]$ in the bathing medium under resting conditions. *Inset* shows a representative A-type current recorded for a step from -100 to -30 mV. Values for V_h and V_a are shown above plots for recordings in control conditions and after perfusion of $0.5 \mu\text{M}$ mibefradil. Blocking T-type current selectively left-shifts K_v4 $V_h \sim -10$ mV ($p < 0.001$) with no effect on V_a ($p > 0.05$) to reduce current amplitude (*inset*). (b, c) Whole-cell recordings of

spike discharge (b) and frequency-current plots (c) from cerebellar lobule 9 granule cells that express the Ca_v3 - K_v4 complex. Recordings were made in a dynamic clamp to reduce K_v4 current online according to measured biophysical properties of Ca_v3 and K_v4 current, with the external level of calcium maintained at 1.5 mM throughout. Dynamic clamp reduction of A-type current increases the rate of spike firing (b) and gain on a frequency-current plot (c). Average values are mean \pm SEM. (Figures are modified from (a) Anderson et al. (2010a), and (b, c) Heath et al. (2014))

further shown that these results could not be reproduced if $K_v4.2$ was coexpressed with any of the HVA classes of calcium channels, revealing the specificity of K_v4 modulation by Ca_v3 channels.

Together these data established that the Ca_v3 - K_v4 complex normally promotes a calcium-dependent rightward shift in K_v4 V_h and thus window current that is centered near resting potential and spike threshold (Fig. 3a, b). Since

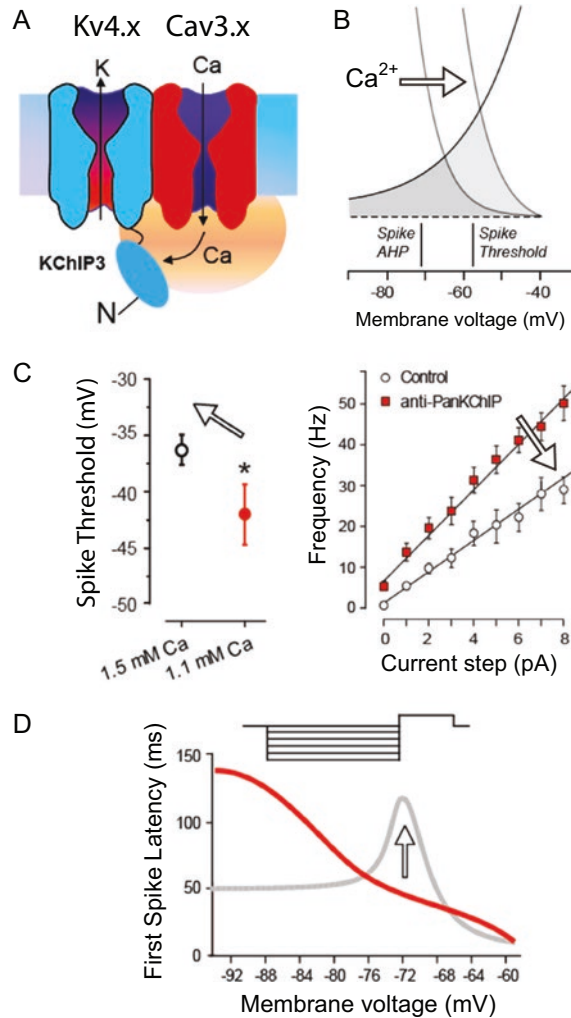


Fig. 3 Ca_v3 calcium influx shifts K_v4 V_h to reduce spike firing and delay first spike latency. **(a)** Cartoon depiction of the Ca_v3-KChIP3-K_v4 complex assembled to function with nanodomain interactions in cerebellar stellate and granule cells. **(b)** Schematic diagram depicting the role of Cav3 calcium influx in normally shifting the K_v4 V_h to the right to increase window current and availability of K_v4 for activation over a voltage range that straddles spike threshold and a fast AHP. *White filled arrow* in this and lower panels depicts the direction of action by the Cav3-K_v4 complex on cell activity. **(c)** Mean scatter plots of the change in spike threshold in stellate cells, and current-

frequency plots of firing rate in granule cells upon interfering with Ca_v3-K_v4 complex function. *White arrows* depict the influence of a Cav3-K_v4 complex on cell function under normal conditions. **(d)** Fits of the mean values for FSL following membrane hyperpolarization in cerebellar stellate cells with (*grey trace*) and without (*red trace*) Ca_v3-K_v4 complex actions. The current step protocol used to activate spike responses to measure FSL following a hyperpolarization is shown above. **p* < 0.05. (Figures are modified from **(a)** Turner and Zamponi (2014), **(b, c)** Anderson et al. (2010b) and Heath et al. (2014), and **(d)** Molineux et al. (2005))

this would change the extent of K_v4 control over the spike threshold, the effects of interfering with the Ca_v3-K_v4 complex on spike output were tested. In stellate cells, a decrease in the level of external calcium from 1.5 to 1.1 mM lowered the

spike threshold to increase the firing rate (Fig. 3c). In cerebellar granule cells, infusing an anti-PanKChIP antibody through the electrode increased spike firing rate gain (Fig. 3c). These data confirmed that the normal role of the Ca_v3-

K_v4 complex is to reduce excitability and the rate of spike firing in both stellate and granule cells. But an additional influence in regulating spike output became apparent in cerebellar stellate cells. One role for A-type current is to modify the latency to first spike discharge following membrane hyperpolarization. First spike latency (FSL) typically takes on a graded, inverse relationship where an increase in the magnitude or duration of a preceding hyperpolarization produces more A-type current upon return to resting potential to delay the firing of the first spike. This is important as FSL represents a key element of encoding membrane hyperpolarizations and sensory input. In these cells, the Ca_v3-K_v4 complex was found to promote an unusual voltage-FSL relationship. Instead of an inverse relationship between membrane hyperpolarization and spike latency, stellate cells instead exhibit a non-monotonic relationship where a delay in FSL was restricted to ~10 mV window of membrane voltage around resting potential (−76 to −65 mV) (Fig. 3d). Reducing Ca_v3 calcium conductance by perfusing low calcium or calcium channel blockers removed the peak of FSL found near resting potentials, revealing a role for Ca_v3 calcium channels in producing this pattern of spike output (Fig. 3d) (Molineux et al., 2005).

Ca_v3.2-IK Complex

Cerebellar Purkinje cells express calcium-gated potassium channels that are activated by the large complex spike response generated by climbing fiber input. HVA calcium channels triggered by climbing fiber input control the duration of the complex spike and a subsequent AHP by activating calcium-gated potassium channels (Schmolesky et al., 2002; Fernandez et al., 2007; McKay et al., 2007; Davie et al., 2008; Ohtsuki et al., 2012; Engbers et al., 2013a; Ait Ouares et al., 2019). It was shown that Purkinje cells also express Cav3 channels and T-type current that can be activated synaptically and during spike discharge (Mouginot et al., 1997; Talley et al., 1999; Swensen & Bean, 2003; Engbers et al., 2012; Isope et al., 2012; Ly et al., 2016). Recently,

work on a parallel fiber EPSP-evoked slow after-hyperpolarization (sAHP) uncovered the additional expression of an intermediate conductance calcium-gated potassium channel (KCa3.1, IK) in Purkinje cells (Engbers et al., 2012). This was surprising in that IK channels were not believed to be expressed in central neurons. Importantly, IK channels are known to be voltage-insensitive and activated by calcium binding to calmodulin (Fig. 1) to evoke a non-inactivating potassium current. But the data of Engbers et al. (2012) revealed that Purkinje cells exhibit a new Ca_v3-IK channel complex (Fig. 4a) formed at the molecular level to effectively convert IK channels to calcium- and voltage-gated A-type channel profile to control several aspects of spike discharge.

Single-pulse stimulation of parallel fibers in the slice preparation established that EPSPs even 5 mV in amplitude are followed by a calcium-dependent sAHP of ~400 msec (Fig. 4b). Pharmacological tests revealed that this sAHP was insensitive to multiple toxins and blockers against the families of HVA calcium channels, including 200 nM AgTx against the P-type calcium channel that is heavily expressed in Purkinje cells. Instead, the Ca_v3 channel blockers Ni²⁺ (100 μM) or mibefradil (1.0 μM) slowed the rate of EPSP decay and blocked the subsequent sAHP (Fig. 4b). In attempting to identify the calcium-gated potassium channel responsible for the sAHP, it became clear that neither SK nor BK channels were involved in the slow AHP in its being insensitive to apamin, iberiotoxin, TEA, or paxilline. Instead, the parallel fiber EPSP rate of decay was reduced, and the slow AHP was blocked by charybdotoxin (ChTx, 100 nM) or the IK-selective blocker TRAM-34 (100 nM) (Fig. 4a, b) (Engbers et al., 2012).

To assess the interaction between Ca_v3 and IK channels they examined the currents evoked in Purkinje cells by a ramp command (500 msec) from a holding potential of −100 to −40 mV to scan from a subthreshold potential to near the peak voltage for T-type current. Ca_v3 current was also pharmacologically isolated using a suite of blockers against sodium, HVA calcium, and potassium channels, including SK, BK, and IK channels. To further identify the Ca_v3-dependent

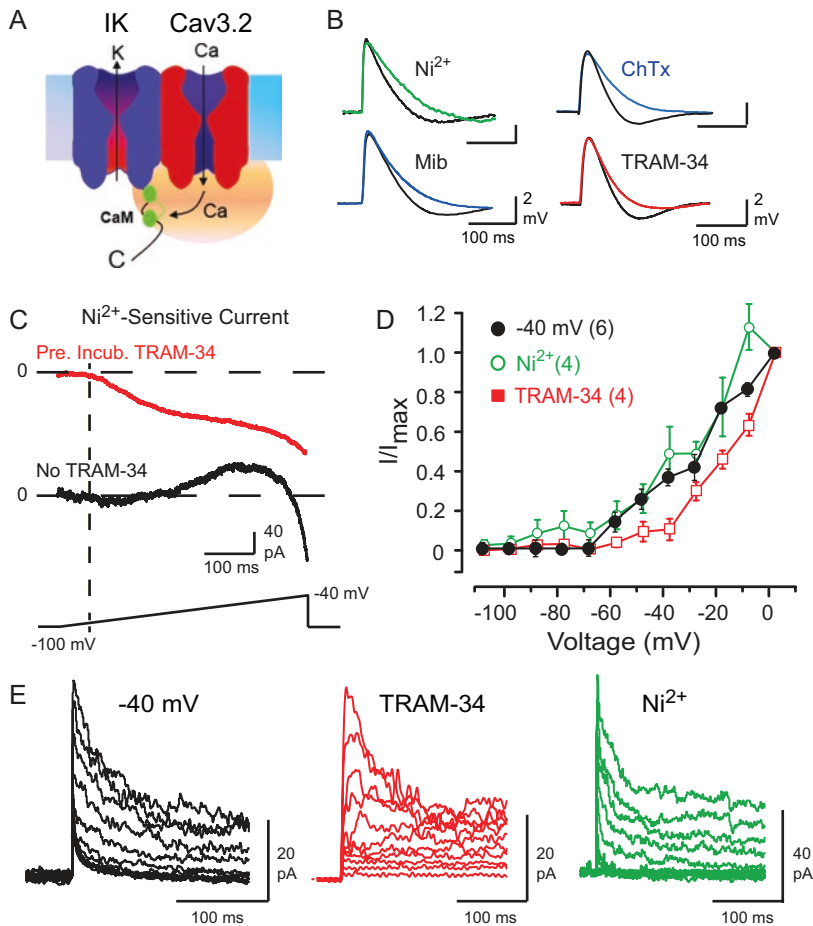


Fig. 4 A $\text{Ca}_v3.2$ -IK channel complex in Purkinje cells imparts low voltage-dependence and an A-type channel profile to IK current. (a) Cartoon depiction of a nanodomain interaction between $\text{Ca}_v3.2$ and IK (KCa3.1) channels. (b) Representative subthreshold simulated parallel fiber EPSPs (simEPSPs) evoked by somatic current injection to test postsynaptic contributions to the generation of a sAHP. Superimposed recordings show a reduction in the decay phase of the simEPSP and sAHP by $100 \mu\text{M}$ Ni^{2+} or $1.0 \mu\text{M}$ mibefradil to block $\text{Cav}3$ calcium channels, or by 100 nM ChTx or 100 nM TRAM-34 to block IK channels. (c) Whole-cell currents evoked by a ramp command under conditions that pharmacologically isolate Ca_v3 current (red trace) or Ca_v3 and IK current (black trace) in the presence or absence of 100 nM TRAM-34 to block IK channels. Ca_v3 -dependent inward or outward currents were

identified by subtracting traces following $100 \mu\text{M}$ Ni^{2+} application. (d, e) Outside-out patch recordings from separate Purkinje cell somata evoked by step commands from -110 to 0 mV under conditions that isolate Ca_v3 and IK current (Engbers et al., 2012). Shown in (d) are mean I-V plots for outward current calculated as the difference from those evoked after a preceding step to -40 mV to inactivate Ca_v3 channels, or those blocked by TRAM-34 (100 nM) or Ni^{2+} ($100 \mu\text{M}$). Shown in (e) are superimposed outward current traces for each of the conditions in (d), revealing a calcium-dependent, low voltage-activated, and fast activating/inactivating IK current. Sample values are shown in brackets in (d) and average values are mean \pm SEM. (Figure is modified from (a) Turner and Zamponi (2014) and (b-e) from Engbers et al. (2012))

component of evoked currents they subtracted from control recordings the current blocked by perfusion of 100 nM Ni^{2+} . These tests uncovered a Ni^{2+} -sensitive inward Ca_v3 calcium current that

was triggered from a voltage of $\sim -90 \text{ mV}$ that grew steadily through the voltage ramp to -40 mV (Fig. 4c). If the same test was repeated in the absence of TRAM-34 to allow activation of

IK channels, Ni^{2+} application instead uncovered a slowly activating net outward current that transitioned into a rapidly increasing inward calcium current as the voltage approached -40 mV (Fig. 4c). These data indicated that the Ca_v3 channel current is sufficient to activate IK channels even at potentials normally subthreshold for spike activation.

To further explore the voltage-dependence for IK activation outside-out patch recordings were obtained from separate Purkinje cells using a step command from -110 to 0 mV under conditions with Ca_v3 and IK currents pharmacologically isolated. Potassium currents were then calculated as the difference from those evoked from a preceding step to -40 mV to inactivate the Ca_v3 current, or after perfusing $100 \mu\text{M}$ Ni^{2+} to block Ca_v3 current or 100 nM TRAM-34 to block IK channels. Plotting potassium currents isolated in this manner revealed a set of I-V relations expected for an LVA outward current over the range of ~ -60 to -0 mV (Fig. 4d). Moreover, superimposing the current traces isolated under each of these conditions revealed a low voltage-activated and fast activating/fast inactivating IK current (Fig. 4e). These results were remarkable in showing that a non-activating calcium-dependent IK channel can be transformed into a low voltage-dependent and rapidly activating/inactivating current by virtue of their association with Ca_v3 calcium channels.

All isoforms of Ca_v3 channels are reportedly expressed in Purkinje cells and with selective expression of one $\text{Ca}_v3.1$ isoform in dendritic spines (McKay et al., 2006; Molineux et al., 2006; Hildebrand et al., 2009; Isope et al., 2012; Ly et al., 2016). Parallel fiber stimulation has also been reported to activate $\text{Cav}3$ calcium current presumably in spines as a significant source of calcium influx given the relative lack of calcium-conducting ligand-gated receptors (Isope & Murphy, 2005; Hildebrand et al., 2009; Isope et al., 2012; Ly et al., 2016). Protein biochemical and immunocytochemical work identified at least the $\text{Ca}_v3.2$ channel isoform as colocalizing and undergoing coimmunoprecipitation with IK channels (Engbers et al., 2012). To assess the influence of the Ca_v3 -IK complex on parallel

fiber-evoked spike output they recorded under voltage clamp the EPSC in Purkinje cell somata in response to direct parallel fiber stimulation. This allowed for subsequent injection of the EPSC waveform through the electrode in other cells to evoke a simulated parallel fiber EPSP as a single response or as a stimulus train (Fig. 5a). In this way the simEPSP was restricted to the postsynaptic cell, allowing bath perfusion of drugs that block $\text{Cav}3$ channels without concern for interfering with presynaptic roles of Ca_v3 channels. Activation of a simEPSP adjusted to just subthreshold for spike discharge as a 25 Hz train in Purkinje cells revealed an initial temporal summation over the first few pulses followed by a gradual decay towards resting membrane potential over the course of 50 pulses (Fig. 5b, c). As a result, the peak EPSP remained below the spike threshold throughout a stimulus train (Fig. 5b, c). However, perfusing $100 \mu\text{M}$ Ni^{2+} or 100 nM TRAM-34 to interfere with the Ca_v3 -IK complex uncovered an EPSP temporal summation that rapidly surpassed the spike threshold throughout the entire stimulus train (Fig. 5b, c). Recordings of this nature thus revealed a key role for the Ca_v3 -IK complex in suppressing temporal summation of postsynaptic parallel fiber EPSPs (Engbers et al., 2012).

Cerebellar Purkinje cells project to postsynaptic cells in the deep cerebellar nuclei or the vestibular nuclei and discharge over a range of frequencies to signal the outcome of signal processing in the cerebellar cortex. It is thus important to ensure faithful propagation of spike discharge down Purkinje cell axons. This requires the coordinated activation of sodium and potassium channels to repolarize spikes to prevent their inactivation at high-frequency rates. The finding of a Ca_v3 -IK complex in Purkinje cells led to a sophisticated set of tests of its potential role in maintaining spike propagation past nodes of Ranvier in Purkinje cell axons (Grundemann & Clark, 2015). Previous work had established that Purkinje cells faithfully transmit spike discharge down the axon at frequencies up to ~ 250 Hz, including branch points where nodes of Ranvier have been localized (Khaliq et al., 2003; Khaliq & Raman, 2005; Monsivais et al., 2005).

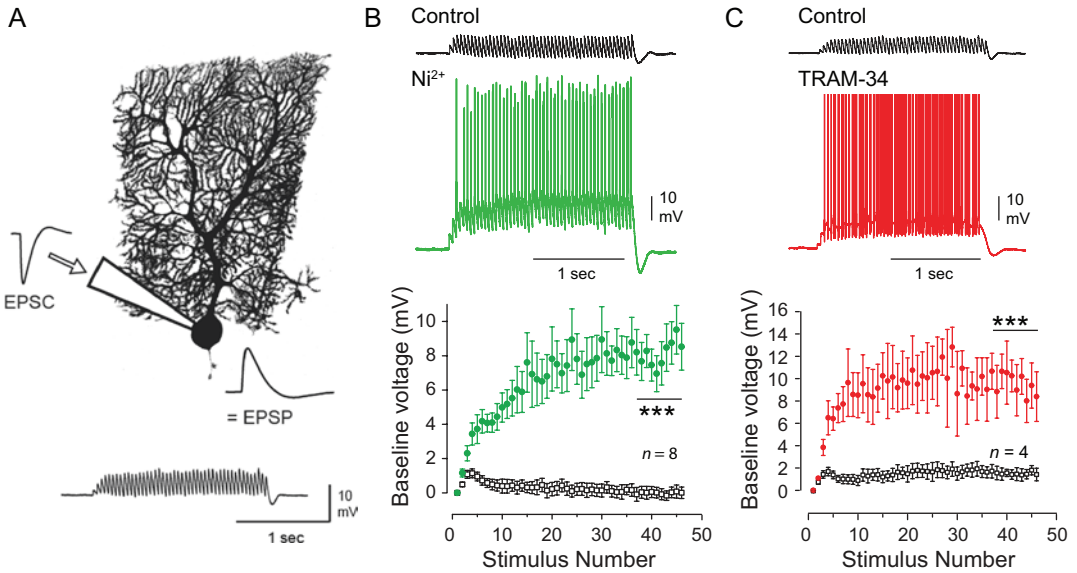


Fig. 5 The Ca_v3 -IK complex suppresses temporal summation to reduce somatic spike firing in Purkinje cells. (a) Schematic diagram of a Purkinje cell and generation of a simulated parallel fiber EPSP by injecting the EPSC recorded in another cell under voltage clamp in response to direct parallel fiber stimulation. Lower trace, a compiled train of simEPSPs generated at 25 Hz and subthreshold for firing to inject into Purkinje cell somata. (b, c) Whole-cell recordings from two separate Purkinje cells of

the response to a train of simEPSPs injected at the soma (Control) and after perfusion of $100 \mu\text{M Ni}_2^+$ (b) or 100 nM TRAM-34 (c). The mean values of baseline voltage preceding each simEPSP are plotted below for control and drug-treated conditions. Spikes were truncated in (c). Average values are mean \pm SEM. Statistical significance was tested for the last 10 pulses of stimulus trains in (b, c). *** $p < 0.001$. (Figure is modified from Engbers et al. (2012))

Using whole-cell recordings at Purkinje cell somata to dye fill the axon, a second on-cell recording could be obtained beyond an axonal branchpoint and node of Ranvier (Fig. 6a) (Grundemann & Clark, 2015). Successful transmission of spontaneous spike discharge at the soma could then be monitored in the axon beyond the node of Ranvier while disrupting the Ca_v3 -IK complex. These tests showed that focal perfusion of $100 \mu\text{M Ni}_2^+$ or $1 \mu\text{M TRAM-34}$ near the branchpoint led to a rapid failure of spike propagation past the axon branchpoint without apparent effect on the baseline rate of somatic spike firing (Fig. 6b, c). Surprisingly, similar applications of apamin or iberiotoxin to block BK or SK channels, or even TEA at 10 mM did not induce failure of spike propagation despite the expected block by TEA of BK, K_v1 , K_v3 , and K_v7 channel subtypes.

Immunocytochemistry revealed IK immunolabel over extended lengths of Purkinje cell axons

without apparent specific localization to nodes of Ranvier. To assess the distribution of Ca_v3 channel activation Grundemann and Clark (2015) used 2-photon imaging of calcium influx to detect increases in intracellular calcium during trains of evoked spikes, which proved to be centered within $5 \mu\text{m}$ of the nodes of Ranvier (Fig. 6d). The change in intracellular calcium was activity-dependent and cumulative in proportion to the rate of spike discharge at the Purkinje cell soma, with a decrease in nodal calcium signal upon somatic hyperpolarization (Fig. 6e). The calcium increase at nodes of Ranvier was further reduced by a low calcium medium or mibefradil but not by the P-type channel blocker AgTx. Further modeling studies suggested that the Ca_v3 -IK interaction is sufficient to sustain spike conduction in Purkinje cell axons.

The work on Purkinje cells thus established the presence of a $\text{Ca}_v3.2$ -IK channel complex where Ca_v3 channel activation imparts a low

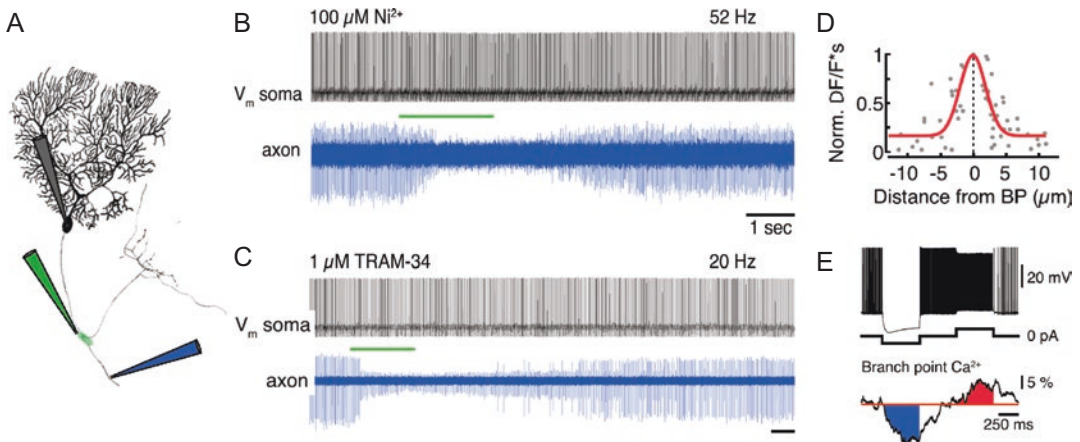


Fig. 6 The Ca_v3 -IK complex ensures axonal spike propagation at Purkinje cell axon nodes of Ranvier. (a) Schematic diagram of a Purkinje cell with a simultaneous whole-cell recording at the soma (grey), a cell-attached axon recording site (blue), and drug ejection electrode positioned near a node of Ranvier and axonal branch site (green). (b, c) Simultaneous whole-cell somatic and cell-attached axon recording of a spontaneously firing Purkinje cell before and after ejection of Ni^{2+} (b) or TRAM-34 (c) (green bar), as per recording configuration shown in (a).

Baseline Purkinje cell firing rate is indicated above somatic traces. (d) Normalized integrated DF/F ($\text{DF}/\text{F}^2\text{s}$) of calcium increases against distance from an axonal branchpoint reveals calcium transients highly localized around the node of Ranvier. (e) Step changes in current injected at a somatic whole-cell recording site modulates spike firing frequency and the corresponding DF/F measured calcium transients at a distant node of Ranvier/axon branchpoint reveal activity-dependent changes in DF/F . (Figure is modified from Grundemann and Clark (2015))

voltage-activation of IK channel current, and with fast activating, fast inactivating kinetics. The end result is to allow Ca_v3 channels to be activated even by parallel fiber input to generate a sAHP that modulates temporal summation during repetitive inputs. Through specific localization of Ca_v3 -IK influence at nodes of Ranvier, this complex further acts as a key factor in maintaining spike propagation down the axons of the principal output neuron of the cerebellar cortex.

$\text{Ca}_v3.2$ -BK Complex

Another calcium-gated potassium channel with a large conductance of up to 200 pS (big conductance, BK, KCa1.1) is distinct from either Kv4 or IK channels in exhibiting seven transmembrane domains and structural features of a voltage-gated channel (Fig. 1). The N-terminus is positioned on an S0 subunit that faces the extracellular space, and an internal C-terminus contains an RCK domain for calcium sensing (Fig. 1). BK channels are responsive to both internal calcium

and membrane voltage in a synergistic manner, typically responding to relatively higher levels of internal calcium increases (1–10 μM) and activating near -20 mV as a non-inactivating current (Womack & Khodakhah, 2002; Latorre & Brauchi, 2006; Berkefeld et al., 2010; Lee & Cui, 2010). As a result, BK channels are expected to be activated by spike-associated depolarizations to contribute to spike repolarization and generation of a fast AHP, with specific interactions with $\text{Ca}_v2.1$ (P/Q-type) and $\text{Ca}_v2.2$ (N-type) calcium channels (Shao et al., 1999; Smith et al., 2002; Womack & Khodakhah, 2002; Sun et al., 2003; Goldberg & Wilson, 2005; Berkefeld et al., 2006; Berkefeld & Fakler, 2008). An association between an HVA calcium channel and BK channels also extends to the lower voltage-activated $\text{Ca}_v1.3$ channel isoform in dopaminergic neurons and chromaffin cells (Marcantoni et al., 2010; Vandael et al., 2010).

In the brainstem vestibular nuclei, BK channel activation has been associated with modifying the gain of the vestibular ocular reflex (VOR) (Smith et al., 2002). This is relevant to cerebellar

function in that Purkinje cells of caudal cerebellar lobules project directly to cells in the medial vestibular nucleus (MVN). Interestingly, a potential association between the LVA $\text{Ca}_v3.2$ calcium and BK channels had been suggested in detecting coimmunoprecipitation between these proteins in brain lysates (Chen et al., 2003). A functional coupling in MVN neurons had further been implicated in a report that the BK-mediated AHP was sensitive to the block of Ca_v3 conductance by Ni^{2+} application (Smith et al., 2002). Recent work now establishes that $\text{Ca}_v3.2$ channels also form a close association with BK channels at the molecular level to invoke LVA activation and fast inactivation of BK outward current (Rehak et al., 2013).

The interactions between $\text{Ca}_v3.2$ and BK channels were investigated using tsA-201 cells and in MVN cells of rat tissue slices *in vitro*. Here it was found that expressing the BK channel alpha subunit alone in tsA-201 cells resulted in a small amplitude non-inactivating outward current in response to a step from -100 to $+40$ mV (Fig. 7a). To test the potential influence of $\text{Ca}_v3.2$ calcium influx, $\text{Ca}_v3.2$ and BK channels were coexpressed (Rehak et al., 2013). This process alone proved to be insufficient to promote any further activation of BK current using a step to $+40$ mV (250 msec). Yet, adding a second step command to $+40$ mV (250 msec interval) that was immediately preceded by a step to -30 mV to activate $\text{Ca}_v3.2$ calcium conductance evoked a BK current 2.7 fold greater in amplitude (Fig. 7a). Moreover, the BK current exhibited a rapid rate of decay ($\tau = 117$ msec) over the 250 msec pulse duration (Fig. 7a). Current density-voltage plots further showed a dramatic leftward shift in the activation voltage of BK current (Fig. 7b). By comparison, these effects were not found if BK channels were coexpressed with a $\text{Ca}_v3.2$ pore mutant that could not conduct calcium (Fig. 7a). Comparing the $\text{Ca}_v3.2$ current evoked by a series of preceding step commands and the P2/P1 ratio for BK current revealed a very close correspondence between the voltage-dependence and magnitude of BK current to that of Ca_v3 current over a range of -90 to $+20$ mV (Fig. 7c). Inspection of the BK or $\text{Ca}_v3.2$ current by calculating the dif-

ference currents after applying Ca_v3 or BK blockers showed that the close relationship between $\text{Ca}_v3.2$ and BK activation extended to kinetic properties. Specifically, when $\text{Ca}_v3.2$ and BK channels were coexpressed BK current adopted a fast activation, fast inactivation profile characteristic of the LVA T-type Ca_v3 current (Fig. 7d). A closer examination of the initial voltage for activation of either current was gained using a ramp command and pharmacological isolation of either current (Fig. 7e). With this, it was apparent that the initial activation of $\text{Ca}_v3.2$ current at ~ -70 mV was very closely associated with the initial voltage for activation of BK current. This is important in revealing a substantial shift in BK activation voltage that will allow it to contribute to both LVA and HVA-related cellular events. By using current-clamp recording conditions in MVN cells *in vitro*, the application of mibefradil reduced both the rate of spike repolarization and the amplitude of the subsequent fast AHP (Fig. 7f), increasing the gain of firing frequency output by 1.4 times (Rehak et al., 2013).

Together these data indicate that the characteristics of BK current were closely linked to the activation voltage, time-course, and rate of inactivation of $\text{Ca}_v3.2$ calcium channels as the source of internal calcium concentration increases. The result was to effectively convert a BK channel current from a non-inactivating and relatively high voltage-activated current to one more characteristic of an LVA A-type potassium current.

Perspectives and Conclusions

The existence of an A-type potassium channel current phenotype has long been recognized for its unique biophysical properties of low voltage for activation and fast activation/inactivation that underlies its important role in regulating spike output. While the expression of an A-type current has been considered almost ubiquitous in central neurons, it was seemingly represented by only a small cohort of isoforms across 3 of the 4 most common voltage-gated potassium channel families ($\text{K}_v1.4$, $\text{K}_v3.4$, and $\text{K}_v4.1-3$). This chapter has highlighted how Ca_v3 channels can effectively

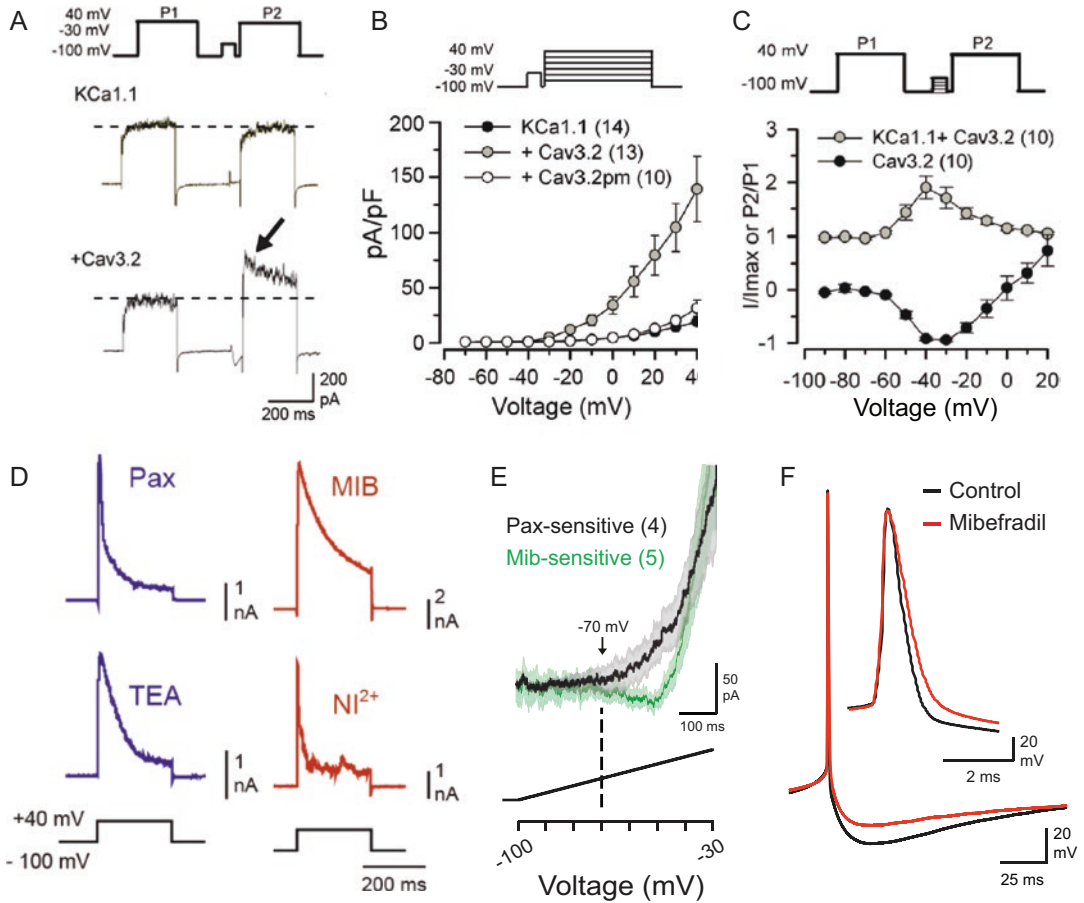


Fig. 7 A $Ca_v3.2$ -BK channel complex in medial vestibular neurons imparts low voltage-dependence and an A-type channel profile to BK current. **(a)** Whole-cell recordings of BK current in tsA-201 cells with or without coexpression of $Ca_v3.2$. BK channel activity was tested using two 250 msec steps to +40 mV (P1, P2) with a 50 msec prepulse to -30 mV immediately prior to P2 to activate Ca_v3 current. In the presence of $Ca_v3.2$ BK activation is facilitated on P2 (*arrow*) by the prepulse used to maximally activate $Ca_v3.2$ current. **(b)** Average current-voltage plots of BK current on P2 indicate that a prepulse to -30 mV in the presence of Cav3 channels significantly left-shifts activation voltage and increases BK current. BK activation is unaltered when coexpressed with a non-conducting calcium channel mutant ($Ca_v3.2$ pm). **(c)** Plots of whole-cell current in tsA-201 cells for $Ca_v3.2$ expressed in isolation and the P2/P1 ratio of BK current as a function of pre-pulse command voltage stepped in 10 mV incre-

ments from -90 to +20 mV. Note the close correspondence in voltage dependence and magnitude of $Ca_v3.2$ and BK currents. **(d)** Whole-cell recordings from MVN cells in vitro under conditions that pharmacologically isolate Ca_v3 and BK channel activity. Shown are difference currents in separate cells after selective block of BK current by 1 mM paxilline (Pax) or 1 mM TEA, and block of Ca_v3 channels by 1 μ M mibefradil (MIB) or 300 μ M Ni^{2+} . All recordings included 30 μ M Cd^{2+} to prevent calcium conductance through HVA calcium channels. **(e)** Mean I-V plots of different currents in MVN cells recorded in response to a ramp command to identify paxilline-sensitive BK current or mibefradil-sensitive Ca_v3 current. **(f)** Current clamp recordings of spike discharge in MVN cells show a reduction of spike repolarization (*inset*) and a subsequent fast AHP by 1 μ M mibefradil. Average values are mean \pm SEM, shown as *shaded regions* on current traces in **(e)**. (Figure is modified from Rehak et al. (2013))

create calcium-dependent A-type current in three additional classes of potassium channels expressed in different cerebellar cell types. By

forming a close association at the molecular level, one can detect an effective transfer of biophysical properties from Ca_v3 channels to each of

K_v4, IK, and BK potassium channel isoforms. These different potassium channels then function effectively as calcium-dependent A-type potassium channels with the rich set of characteristics inherent to a fast activating, fast inactivating channel. Thus, the voltage-dependent class of K_v4 channels acquires a calcium dependence through Ca_v3 calcium interaction with KChIP3 to regulate cell excitability in stellate and granule cells. The IK channel in Purkinje cells instead transforms from a calcium-dependent but a voltage-independent channel to one that exhibits a very low voltage for activation with fast inactivation to modulate synaptic input and spike output. Finally, pairing Ca_v3.2 channels with the voltage-dependent but non-inactivating BK channel imparts a low voltage for activation and fast inactivation to control the gain of spike firing by modulating spike repolarization and a fast AHP in MVN neurons.

The current review focused on the effects of these channel interactions on spike output. But the existence of these Ca_v3-K channel pairings extends well beyond this to other aspects of signal processing. For instance, the influence of a Ca_v3-K channel coupling on cell activity can differ even within even a single cell according to regional localization. Thus, the Ca_v3-IK interaction in the soma of Purkinje cells produces a sAHP that reduces the temporal summation of parallel fiber input. In contrast, the same interaction centered on the nodes of Ranvier instead ensures faithful propagation of sodium spikes over Purkinje cell axons. The Ca_v3-K_v4-KChIP3 complex proves to be highly sensitive to shifts in external calcium concentrations during repetitive climbing fiber input that reduces external calcium. The reduction in external calcium (~0.4 mM) then lowers Ca_v3 calcium conductance to promote the left shift in K_v4 V_h to increase the rate of stellate cell firing (Anderson et al., 2013). In granule cells, a Ca_v3 calcium-induced shift in K_v4 V_h can be induced on a long-term basis through an ERK-dependent process to promote long-term potentiation of granule cell excitability to mossy fiber input (Rizwan et al., 2016). The influence of the Ca_v3-K_v4-KChIP3 complex on signal processing is further deter-

mined by the relative expression pattern of each of these subunits across cerebellar lobules. Thus, a high density of Ca_v3.1 and KChIP3 expression in lobule 9 tunes granule cells to respond to oscillatory-like signals related to vestibular input. But a lower expression of Ca_v3 channels in lobule 2 granule cells reduces the effectiveness of the Ca_v3-K_v4 complex to instead select for burst-like patterns of mossy fiber input (Heath et al., 2014). Since these subunits exhibit different expression patterns in various brain regions the role of Ca_v-K_v4 interactions could well be cell-specific or adjusted to produce different outcomes in the same class of cells.

K_v4, IK, and BK channels differ in terms of the calcium sensor and thus relative sensitivity to changes in calcium concentration. The N-terminus of K_v4 channels is known to complex with KChIP3, IK channels bind calmodulin at the C-terminus, while BK channels employ an RCK domain on the C-terminus (Fig. 1). Nanometer proximity of Ca_v3 channels with K_v4 and IK channels that incorporate the relatively sensitive KChIP3 and calmodulin as calcium sensors ensure rapid responses to changes in cell activity. Interestingly, the RCK domain of BK channels can require elevations of intracellular calcium in the order of 1–10 μM before modulating channel activity. The relatively low conductance and fast inactivation of Ca_v3 channels reduces the domain of calcium increase associated with channel opening. As a result, interesting interactions can occur in which virtually no effect is detected on BK channel properties with simple coexpression of Ca_v3.2 (Fig. 7a). Yet providing a prepulse to initiate Ca_v3 calcium influx immediately before activating BK channels reveals a distinct influence by imparting a low voltage for BK channel activation. This interaction thus provides the ability to participate in cell functions at membrane voltages not previously achieved even with the reciprocal interaction between HVA calcium influx and voltage inherent to BK channels. Modeling to investigate this process revealed that it reflects a microdomain interaction that requires the concerted actions of multiple Ca_v3 channels (Engbers et al., 2013b; Rehak et al., 2013). The functions enabled by an association between

Ca_v3.2 and BK channels are thus distinctly different from that found for coupling to HVA Ca_v1.2 and Ca_v1.3, Ca_v2.1 and Ca_v2.2 channels (Prakriya & Lingle, 1999; Grunnet & Kaufmann, 2004; Berkefeld et al., 2006; Loane et al., 2007; Berkefeld & Fakler, 2008).

The full slate of potassium channel subtypes that might be modulated by colocalizing Ca_v3 calcium and potassium channels remains to be determined. The current review focused only on cell types within the cerebellum and one of its primary output targets in the MVN. Ca_v3 channels have also been shown to at least functionally couple to SK calcium-dependent potassium channels in Purkinje cells as well as dopaminergic midbrain neurons (Wolfart et al., 2001; Wolfart & Roeper, 2002; Cueni et al., 2009; Ait Ouares et al., 2019). But the extent to which this reflects a direct interaction at the molecular level to impart Ca_v3 channel LVA properties and fast inactivation on SK channels is not fully known. The widespread expression of A-type potassium current in cells across the CNS speaks to the importance of this phenotype of potassium channel output. The ability for Ca_v3 calcium channels to effectively generate 3 new forms of A-type potassium channels that are both calcium- and voltage-dependent will substantially increase the range of functions that can be realized by A-type potassium currents.

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