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Regulation of the KV4.2 complex by CaV3.1 calcium channels

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A-type potassium current generated by the KV4 family of channels is an important factor regulating the frequency, latency and dendritic backpropagation of spike discharge. The KV4.2 complex of KV4.2-KChIP3-DPP10c was recently shown to form a novel signaling complex through its association with T-type CaV3.2 or CaV3.3 calcium channel isoforms. CaV3-mediated calcium entry was shown to selectively right-shift the inactivation voltage of KV4.2 into the physiological range to modulate cerebellar stellate cell latency and gain. We now show that CaV3.1 calcium channels can also associate with the KV4.2 complex to effect similar regulation of KV4.2 inactivation. By comparison, no calcium-dependent shift in KV4.2 inactivation properties was elicited by any of CaV1.4, CaV2.1 or CaV2.3 calcium channels coexpressed with the KV4.2 complex, emphasizing the important role for low voltage-activated CaV3 channels in this signaling complex.

Introduction

Action potential discharge is highly regulated by inward and outward currents that activate at subthreshold membrane potentials. Two currents that operate in this range arise from transiently activated A-type potassium channels of the KV4 family and CaV3 T-type calcium channels. A-type currents (IA) are known to influence first spike latency, spike frequency and the amplitude of backpropagating dendritic spikes.1-6 T-type currents (IT) have a wide range of functions based on their immediate depolarizing action on membrane potential as well as second messenger regulation of biochemical pathways.7-10 We recently determined that CaV3 and KV4 channels form a signaling complex that allows for calcium-dependent regulation of KV4 inactivation.11 Specifically, T-type mediated calcium influx in cerebellar stellate cells selectively right-shifts the inactivation profile of IA, with no effects on parameters of activation or recovery from inactivation. The function of this complex proves to be critical in shifting the KV4 inactivation profile into the physiological range of membrane potentials to control first spike latency and gain of stellate cell firing.3,11

The KV4 complex is known to contain four K Channel Interacting Proteins (KChIPs1-4) that represent members of a calcium sensor family,5,12 as well as dipeptidyl-peptidase-like-proteins (DPLPs).13-16 Despite a wealth of information on the structural and functional relationships between members of the KV4 complex,13,17,18 little progress had been made on identifying a physiological source of calcium that might modify KV4 function. We found that KV4.2 channels co-immunoprecipitate with CaV3.2 or CaV3.3 channels and are pulled down by GST fusion proteins of the CaV3.2 or CaV3.3 C-terminus.11 Recapitulation of the stellate cell KV4.2 complex in the tsA-201 expression system revealed that KV4 modulation occurs upon co-expression of KChIP3 as a calcium sensor and CaV3.2 or CaV3.3 channels, but not with the high voltage-activated (HVA) CaV2.2 (N-type) calcium channel.11

The tests conducted in Anderson et al.11 focused on a subset of proteins representative of those expressed in cerebellar stellate cells: KV4.2, CaV3.3, KChIP3 and DPP10c.19,20 However, KV4.2 channels might also be subject to regulation.
families of calcium channels are capable of modulating KV4.2 Vh. These tests are also relevant to cerebellar stellate cells in that immunocytochemistry revealed immunolabel for L-type (Ca V1.4), P-type (CaV2.1) and R-type (Ca V2.3) calcium channels (Fig. 2A–C). Voltage-clamp recordings were conducted on tsA-201 cells co-expressing KV4.2, KChIP3, DPP10c and one of these HVA calcium channels to assess their ability to modulate KV4 properties. The expression of HVA calcium channels in each case was confirmed by the presence of inward current during depolarizing pre-potentials or following drug application to block calcium current (data not shown).

Despite the expected overexpression of HVA calcium channels in this assay, application of blockers or toxins specific to each of the HVA channels produced no shift in KV4.2 Vh (Fig. 2A–C).

Discussion

The current study extends the work of Anderson et al.11 on the link between CaV3 calcium channels and the Kv4 potassium channel complex that confers calcium-dependent regulation of I_A. Here we establish that CaV3.1 channels also link to the Kv4.2 complex and can induce a selective modulation of Kv4.2 Vh. These tests are also relevant to cerebellar stellate cells in that immunocytochemistry revealed immunolabel for L-type (CaV1.4), P-type (CaV2.1) and R-type (CaV2.3) calcium channels (Fig. 2A–C). Voltage-clamp recordings were conducted on tsA-201 cells co-expressing CaV4.2, KChIP3, DPP10c and one of these HVA calcium channels to assess their ability to modulate Kv4 properties.

The expression of HVA calcium channels in each case was confirmed by the presence of inward current during depolarizing pre-potentials or following drug application to block calcium current (data not shown). Despite the expected overexpression of HVA calcium channels in this assay, application of blockers or toxins specific to each of the HVA channels produced no shift in CaV4.2 Vh (Fig. 2A–C).
that IA can offset burst capability. A previous study of inhibitory interneurons of rat thalamus also reported that an IT-driven form of burst discharge was attenuated by IA.25 The extent to which this might reflect a simple co-expression and activation of these opposing currents in the same cell or an active shift in IA by CaV3-mediated calcium current remains to be seen.

The expression pattern of CaV3.1 protein immunolabel is also interesting in showing a correlation to cells that have the ability to generate rebound bursts following membrane hyperpolarizations. 19 Burst discharge in thalamic relay cells that express CaV3.1,21,22 is thus blocked in CaV3.1 knockout animals, 26 while all cerebellar cells that express CaV3.1 exhibit rebound bursts under normal conditions.19 In this regard, it is interesting that the extent of the shift in the Vh of KV4 current inactivation by CaV3.1-mediated calcium influx (∼4.5 mV) is approximately half of that produced by CaV3.2 and CaV3.3 (∼7–9 mV).11 It is thus possible that CaV3.1-dominated cell types experience less calcium-dependent shift in Ia Vh than calcium-dependent shift in Kv4 Vh, indicating that this form of modulation can occur between all members of the CaV3 family and Kv4.2 channels. These data further indicate a common site for the association at the CaV3 C-terminus that enables calcium-dependent interaction through KChIP3 proteins. In contrast, co-expression studies now indicate that calcium influx through any of four different HVA calcium channels (L, N, P, R) cannot modulate Kv4.2 inactivation even when overexpressed in tsA-201 cells (Fig. 2).11 The selective ability for CaV3 channels to modulate Ia at the nanodomain level even when each of these HVA calcium channels are expressed in these small diameter cells supports the specificity of the association between CaV3 and Kv4.2 ion channels. Indeed, members of this signaling complex seem well suited given the distinctive but similar activation/inactivation profiles of T-type and A-type channels, allowing calcium entry to closely match the activity of A-type potassium channels even at subthreshold potentials.

The finding that the Kv4.2 complex can associate with CaV3.1 channels, as well as with CaV3.2 and 3.3 isoforms, greatly increases the potential for modulation of Ia, as CaV3 channel isoforms are differentially distributed in CNS neurons. In situ hybridization studies first established a differential distribution for CaV3 mRNA expression,21-23 while immunocytochemical studies of CaV3 channel isoforms indicated that most cells exhibit immunolabel for at least two if not all three of the CaV3 isoforms.19,24 However, specific expression patterns can be found, with a combination of CaV3.2 and CaV3.3 but not CaV3.1 in cerebellar stellate cells.19 This compares to a prominent CaV3.1 label in some cells (i.e., Transient Burst deep cerebellar cells, lateral habenular neurons) to cases in which only CaV3.1 immunolabel is detected (i.e., cerebellar Golgi cells).19,24 In the case of cerebellar stellate cells, we found that the calcium-dependent shift in Vh establishes a novel voltage-first spike latency relationship.3 Blocking Ia in these cells revealed the capacity to otherwise generate rebound frequency increases in firing, demonstrating that Ia can offset burst capability. A previous study of inhibitory interneurons of rat thalamus also reported that an Ia-driven form of burst discharge was attenuated by Ia.25 The extent to which this might reflect a simple co-expression and activation of these opposing currents in the same cell or an active shift in Ia Vh by CaV3-mediated calcium current remains to be seen.

The expression pattern of CaV3.1 protein immunolabel is also interesting in showing a correlation to cells that have the ability to generate rebound bursts following membrane hyperpolarizations.19 Burst discharge in thalamic relay cells that express CaV3.1,21,22 is thus blocked in CaV3.1 knockout animals,26 while all cerebellar cells that express CaV3.1 exhibit rebound bursts under normal conditions.19 In this regard, it is interesting that the extent of the shift in the Vh of Kv4 current inactivation by CaV3.1-mediated calcium influx (∼4.5 mV) is approximately half of that produced by CaV3.2 and CaV3.3 (∼7–9 mV).11 It is thus possible that CaV3.1-dominated cell types experience less calcium-dependent shift in Ia Vh than
those with prominent CaV3.2 or CaV3.3 expression. We also found a pattern in the relative distribution of immunolabel for CaV3 channel isoforms over the soma-dendritic axis of many central neurons. In general, CaV3.1 immunolabel was predominantly located near the soma, CaV3.2 immunolabel over the soma and proximal dendritic regions, and CaV3.3 over most of the soma-dendritic axis. The possibility then exists for the subcellular distribution of CaV3 calcium channel isoforms in somatic and dendritic regions to exert differential control of membrane excitability through \( I_n \). 

It is important to stress that calcium-dependent modulation of \( I_n \) by this signaling complex requires the co-expression of not only CaV3 and K\(_A\) channels but also KChIP3 as the critical calcium sensor. Nevertheless, it is not currently known if co-expression of these proteins guarantees a functional association as a CaV3-K\(_A\) signaling complex. Moreover, CaV3 and K\(_A\) channels may not always be co-expressed within the same neuron. For instance, deep cerebellar nuclear neurons label differentially for CaV3.1 or CaV3.3 channels but neither express an A-type potassium current. The pattern of expression for the K\(_A\) current and that of KChIP3 also exhibit greater variation than that of CaV3 channels. In particular, the brain regions for which this signaling complex controls neuronal activity will presumably show a dependence on the distribution of KChIP3.

Finally, we have not assessed whether other sources of calcium influx are capable of regulating K\(_A\) \( V_h \) through KChIP3. Thus, it is possible that calcium influx through NMDA or AMPA receptors, mGluRs, TRP channels, or calcium release from internal stores are capable of controlling membrane excitability through the CaV3-K\(_A\) signaling complex.

**Materials and Methods**

Co-immunoprecipitation and GST pull-down assays. Co-immunoprecipitation and GST pull-down assays were performed as described in Anderson et al.  

**Immunocytochemistry.** Immunocytochemical sections were prepared as described in Anderson et al. 

Polyclonal primary antibodies were rabbit polyclonal anti-CaV1.4 (1:2,000; gift of T. Snutch, Vancouver, BC), anti-CaV2.1 (1:50; Alomone, Jerusalem), and anti-CaV2.3 (1:100; Sigma-Aldrich, Oakville, ON) applied overnight at 4°C in a working solution of 3% normal donkey serum (Jackson, West Grove, PA), 0.1% TWEEN, and 1% DMSO in phosphate buffer. Anti-MAP-2 antibodies were monoclonal MAP-2 (1:1,000) or polyclonal MAP-2 (1:250) (AbCam, Cambridge, MA). Secondary antibodies (1:1,000) were Alexa Fluor-488 or -594 conjugated donkey or goat anti-rabbit or anti-mouse IgGs (Molecular Probes, Eugene, OR). Immunoreactivity was assessed using a Zeiss AxioImager microscope and Apotome grid illumination system. All immunoblot data and immunocytochemistry shown is representative of 2–3 separate experiments.

**tsA-201 cell electrophysiology.** cDNA for a representative K\(_A\) complex of K\(_A\)2.2, KChIP3 and DPP10c was co-transfected in tsA-201 cells as previously described. cDNA (2.5 µg/µl) for CaV3.1, CaV1.4, CaV2.1, CaV2.3 and the associated β1b and α2-δ subunits were cotransfected with those of the representative K\(_A\) complex and K\(_A\)2.2 current recorded at room temperature. Mibebradil (0.5 µM) and nifedipine (5 µM) were bath applied and ω-agatoxin IVA (200 nM) were focally ejected in a carrier medium containing (in mM): 120 NaCl, 3 NaHCO3, 4.2 KCl, 1.2 KH2PO4, 1.5 MgCl2, 10 Glucose, 10 HEPES, 1.5 CaCl2. Steady-state K\(_A\) inactivation was determined from a carrier medium containing (in mM): 120 NaCl, 3 NaHCO3, 4.2 KCl, 1.2 KH2PO4, 1.5 MgCl2, 10 Glucose, 10 HEPES, 1.5 CaCl2. 

**References**


