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Dominant-negative suppression of $Ca_v2.1$ currents by $\alpha_1 2.1$ truncations requires the conserved interaction domain for β subunits

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Episodic ataxia type 2 (EA2) is an autosomal dominant disorder arising from *CACNA1A* mutations, which commonly predict heterozygous expression of $Ca_v2.1$ calcium channels with truncated $\alpha_1 2.1$ pore subunits. We hypothesized that $\alpha_1 2.1$ truncations in EA2 exert dominant-negative effects on the function of wild-type subunits. Wild-type and truncated $\alpha_1 2.1$ subunits with fluorescent protein tags were transiently co-expressed in cells stably expressing Ca_v auxiliary β subunits, which facilitate α_1 subunit functional expression through high-affinity interactions with the alpha interaction domain (AID). Co-expression of wild-type subunits with truncations often resulted in severely reduced whole-cell currents compared to expression of wild-type subunits alone. Cellular image analyses revealed that current suppression was not due to reduced wild-type expression levels. Instead, the current suppression depended on truncations terminating distal to the AID. Moreover, only AID-bearing $\alpha_1 2.1$ proteins co-immunoprecipitated with Ca_v β subunits. These results indicate that Ca_v β subunits may play a prominent role in EA2 disease pathogenesis.

Keywords: *CACNA1A*; P/Q-type; Channelopathy; Cerebellum; Stress; Ca_v β subunit

Introduction

The dominantly inherited paroxysmal disorder episodic ataxia type 2 (EA2) results from mutations in the *CACNA1A* gene, which encodes the pore-forming $\alpha_1 2.1$ subunit of $Ca_v2.1$ voltage-gated calcium channels (Ophoff et al., 1996). The $Ca_v2.1$ calcium channel subtype regulates neurotransmission throughout the nervous system, but is predominantly expressed within cerebellar Purkinje cells (Usowicz et al., 1992; Stea et al., 1994; Westenbroek et al., 1995). Not surprisingly, cerebellar dysfunction is the primary feature of EA2 attacks as patients experience bouts of symptoms such as ataxia, migraine and vertigo, in response to emotional, physical or pharmacological stressors (Ptacek, 1998; Jen, 2000; Jen et al., 2004). Although episodic neurological disorders can be characterized by a wide range of symptoms, including epileptic seizures, paroxysmal dyskinesias or periodic paralysis, many also arise from mutations within ion channel genes (Jen, 1999; Ptacek, 1999). Thus, studying the pathophysiological mechanisms of individual diseases such as EA2 may be useful in the development of treatment strategies for episodic channelopathy disorders in general.

Voltage-gated calcium or Ca_v channels regulate an array of physiological processes including muscle contraction, hormone secretion, neurotransmission and gene expression. This diversity in function is mainly due to the expression of 10 genetically distinct α_1 subunit subtypes that may make up the calcium channel pore (Ertel et al., 2000; Catterall et al., 2005). However, high voltage-activated (HVA) Ca_v channels are also composed of at least two auxiliary subunits, β and $\alpha_2\delta$, which modulate channel kinetics (Singer et al., 1991; Arikkath and Campbell, 2003; Catterall et al., 2005). The auxiliary β subunits also play a crucial role in the functional expression of Ca_v channels. These non-membrane-spanning subunits promote translocation of α_1 subunits from the endoplasmic reticulum (ER) to the plasma membrane through a high-affinity association with the alpha interaction domain, AID, which is evolutionarily conserved in all α_1 subunit subtypes (Pragnell et al., 1994; De Waard et al., 1996; Bichet et al., 2000).

Functional expression studies involving EA2 mutations have firmly established that non- or hypo-conductive $\alpha_1 2.1$ subunits cause the disorder (Guida et al., 2001; Jen et al., 2001; Jouvenceau et al., 2001; Wappl et al., 2002; Imbrici et al., 2004; Spacey et al., 2004; Imbrici et al., 2005; Wan et al., 2005b; Jeng et al., 2006), which is largely but not exclusively associated with expression of $\alpha_1 2.1$ truncation mutants (Ophoff et al., 1996; Yue et al., 1998; Battistini et al., 1999; Denier et al., 1999; Jen et al., 1999, 2004;

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Denier et al., 2001; van den Maagdenberg et al., 2002; Wappl et al., 2002; Subramony et al., 2003; Mantuano et al., 2004; Eunson et al., 2005; Spacey et al., 2005; Wan et al., 2005a,b; Scoggan et al., 2006). However, the molecular mechanisms by which non-functional $\alpha_1$2.1 pores generate disease in EA2 are still debated. Although some studies have suggested that the loss of channel function in EA2 simply induces a haplo-insufficiency of Ca_v2.1 currents (Wappl et al., 2002; Imbrici et al., 2004, 2005), substantial evidence argues that non-conductive $\alpha_1$2.1 mutants in EA2 actually suppress the functional contributions of Ca_v2.1 channels composed of wild-type subunits through a dominant-negative mechanism (Jouvenceau et al., 2001; Raghib et al., 2001; Arikkath et al., 2002; Page et al., 2004; Jeng et al., 2006). Studies have suggested that impaired translation or stability of wild-type $\alpha_1$2.1 subunits contributes to EA2 pathogenesis (Raghib et al., 2001; Page et al., 2004), while other evidence implicates the interactions between non-conductive $\alpha_1$2.1 mutants and auxiliary β subunits in the dominant-negative suppression of wild-type $\alpha_1$2.1 subunit function (Arikkath et al., 2002; Jeng et al., 2006).

The lack of a clear disease model for EA2 may be due in part to important differences between methods of Ca_v channel functional expression in the laboratory. Therefore, to further test the hypothesis that $\alpha_1$2.1 mutants in EA2 exert dominant-negative effects on Ca_v2.1 function, we utilized a strategy designed to reliably record whole-cell Ca_v2.1 currents from channels composed of auxiliary β and $\alpha_2\delta$ subunits and mixed populations of $\alpha_1$2.1 subunits, containing both wild-type isoforms and truncation mutants. We found that non-conductive $\alpha_1$2.1 truncations, including those associated with EA2, severely suppressed Ca_v2.1 currents when co-expressed with wild-type $\alpha_1$2.1 subunits. Current suppression was observed despite abundant expression of wild-type $\alpha_1$2.1 subunits, demonstrating that these effects were not due to wild-type protein instability. Furthermore, of the several $\alpha_1$2.1 truncation mutants tested, only those terminating distal to the AID suppressed Ca_v2.1 currents. These results are consistent with a dominant-negative model of EA2 disease pathogenesis and further implicate the involvement of Ca_v β auxiliary subunits in EA2 pathophysiology.

Results

Truncated $\alpha_1$2.1 subunits suppress Ca_v2.1 currents in a length-dependent fashion

To directly test for a dominant-negative effect of truncated $\alpha_1$2.1 subunits on Ca_v2.1 channel function, we assembled a series of cDNA constructs designed to express wild-type and truncated $\alpha_1$2.1 subunits, N-terminally tagged with cyan or yellow fluorescent protein, respectively (CFP and YFP; Fig. 1A) and *transiently* expressed them in human embryonic kidney cells *stably* expressing Ca_v β and $\alpha_2\delta$ auxiliary subunits (β_1-HEK cells; Piedras-Renteria et al., 2001). Untransfected β_1-HEK cells expressing only the Ca_v auxiliary subunits failed to exhibit currents at any potential (Fig. 1B). In contrast, whole-cell currents from voltage-clamped β_1-HEK cells expressing CFP-fluorescing wild-type $\alpha_1$2.1 subunits (CFP-Wt) were easily obtainable (Figs. 1B, 2) and exhibited a mean peak current density of -75.5 ± 7.9 pA pF^{-1} at 0 mV ($n=16$; Table 1). Consistent with all previous reports, none of the YFP-tagged $\alpha_1$2.1 truncations tested here conducted detectable Ca_v2.1 currents (not shown).

In several cases, co-expression of wild-type and truncated $\alpha_1$2.1 subunits resulted in significantly reduced whole-cell Ca_v2.1 current densities. Interestingly, the density of currents in co-transfected cells depended on the length of truncated isoform co-expressed. Co-expression of CFP-Wt subunits with either of the YFP-612X or YFP-1443X truncations, similar to mutants predicted from two distinct nonsense mutations associated with EA2 (Denier et al., 1999; van den Maagdenberg et al., 2002), resulted in severe reductions in Ca_v2.1 currents across all voltages compared to control (Figs. 1B, 2A). Cells exhibited mean peak current densities

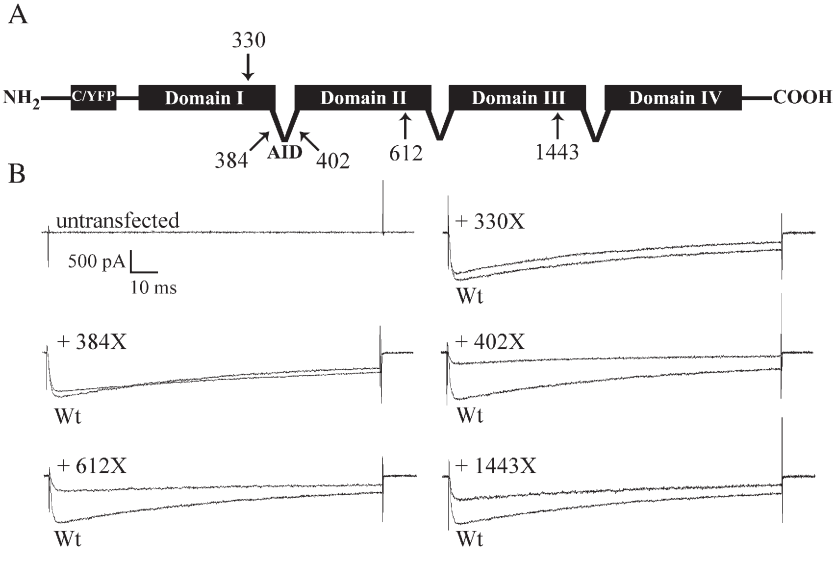

Fig. 1. Design and co-expression of CFP-tagged wild-type $\alpha_1$2.1 subunits with YFP-tagged truncations. (A) Schematic of CFP-tagged wild-type $\alpha_1$2.1 subunits (Wt) and YFP-tagged truncations indicates the amino-acid residue numbers and approximate length of each truncated isoform. The alpha interaction domain, AID, between repeat domains I and II is also marked. (B) Current traces depicted were elicited with 0 mV depolarizing pulses from −80 mV holding potentials following transient co-expression of truncations with Wt subunits in β_1-HEK cells. Representative traces from cells co-expressing truncations with Wt subunits are overlaid with one obtained from a control cell expressing Wt subunits alone.