Journal Club

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The Calcium Channel β_{4a} Subunit: A Scaffolding Protein Between Voltage-Gated Calcium Channel and Presynaptic Vesicle-Release Machinery?

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Review of Vendel et al. (http://www.jneurosci.org/cgi/content/full/26/10/2535)

Neuronal voltage-gated calcium channels represent a major pathway for calcium entry into nerve termini, where they control neurotransmitter release. The channels are composed of a pore-forming subunit ($Ca_v2.x$) and auxiliary subunits ($Ca_v\beta_{1-4}$, $\alpha_2\Delta_{1-4}$ and γ_{1-8}). The cytoplasmic $Ca_v\beta$ subunits belong to the membrane-associated guanylate kinase (GK) family of proteins and regulate trafficking to the plasma membrane and gating properties of $Ca_v2.x$ channels. Their five domains (A–E), include hypervariable A, C, and E domains linked to the highly conserved Src homology 3 (SH3) (B) and GK (D) domains. The crystal structure of the core domains (B–D) of several β subunits was recently elucidated (Chen et al., 2004; Opatowsky et al., 2004; Van Petegem et al., 2004), but less is known about the structure of the A and E domains. A recent report published in *The Journal of Neuroscience* provides new information on the $Ca_v\beta_{4a}$ subunit hypervariable A domain ($Ca_v\beta_{4a}$-A) (Vendel et al., 2006a). The authors previously showed that alternative splicing of the $Ca_v\beta_4$-A domain generates two distinct proteins, $Ca_v\beta_{4a}$ and $Ca_v\beta_{4b}$ (Helton and Horne, 2002), and they recently provided the solution structure of the $Ca_v\beta_{4a}$-A domain (Vendel et al., 2006b). Vendel et al. now address the expression pattern of the $Ca_v\beta_{4a}$ splice variant and the functional importance of the $Ca_v\beta_{4a}$-A domain.

Using immunohistochemistry, the authors show that the $Ca_v\beta_{4a}$ splice variant was expressed as punctuate structures throughout the molecular layer of the cerebellum [Vendel et al. (2006a), their Fig. 3*D*,*E* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F3)]. In contrast, $Ca_v\beta_{4b}$ was expressed in basket cells surrounding Purkinje cell bodies as well as in the Bergmann glia [Vendel et al. (2006a), their Fig. 3*F* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F3)]. To determine the functional importance of the $Ca_v\beta_{4a}$-A domain, the authors performed two electrode voltage-clamp recording in *Xenopus laevis* oocytes expressing $Ca_v2.1$ channels (P/Q type currents) in combination with $\alpha_2\Delta$ and $Ca_v\beta_{4a}$ subunits with (ABCDE) or without (BCDE) the N-terminal A domain. The $Ca_v\beta_{4a}$-A domain was not essential for the expression of the $Ca_v2.1$ channel at the plasma membrane and did not influence gating properties. Indeed, no difference was observed in current amplitude [Vendel et al. (2006a), their Fig. 2*A* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F2)], nor in the voltage dependence of activation or inactivation [Vendel et al. (2006a), their Fig. 2*C*,*D* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F2)]. These results are, however, in agreement with published studies (Bichet et al., 2000; Van Petegem et al., 2004) because (1) enhanced $Ca_v2.x$ subunit trafficking to the plasma membrane occurs after binding of the $Ca_v\beta$ subunit to the channel (Bichet et al., 2000) via the α interaction domain (AID)-binding pocket within the GK domain (Van Petegem et al., 2004), and (2) the molecular determinants by which the $Ca_v\beta$ subunit modulates channel gating remain unclear but are probably carried by the conserved core domains because all $Ca_v\beta$ subtypes are able to modulate the biophysical properties of the channel. However, it is more surprising that channel inactivation kinetics was not influenced by deletion of the $Ca_v\beta_{4a}$-A domain [Vendel et al. (2006a), their Fig. 2*B* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F2)]. Indeed, it is known that a deletion of the N-terminal of $Ca_v\beta$ subunits results in a drastic slowing of channel inactivation kinetics (Olcese et al., 1994). It seems not to be the case for the $Ca_v\beta_{4a}$ splice variant. Thus, further investigations will provide interesting structural information on how the N-terminal of $Ca_v\beta$ subunits controls channel inactivation.

Because the $Ca_v\beta_{4a}$-A domain was not

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a key determinant in the regulation of $Ca_v2.1$ channel gating, Vendel et al. looked for a role of this domain in protein–protein interactions. Using the yeast two-hybrid system, the authors screened a human cerebellum cDNA library with the $Ca_v\beta_{4a}$-A domain (amino acids 1–58). Synaptotagmin I (Syt I) (amino acids 95–337 including the entire C2A domain and a half of the C2B domain) as well as the microtubule-associated protein 1A (MAP1A) (amino acids 2508–2775 corresponding to the complete LC2 domain) interacted specifically with the N-terminal A domain of the $Ca_v\beta_{4a}$ splice variant but not with the $Ca_v\beta_{4b}$-A domain [Vendel et al. (2006a), their Fig. 4*B* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F4)]. The authors then focused their study on Syt I and confirmed its interaction with the $Ca_v\beta_{4a}$-A domain by *in vitro* pull-down experiments. The interaction did not occur in the presence of 10 mM Ca^{2+} and could also be disrupted by adding Ca^{2+} to the medium [Vendel et al. (2006a), their Fig. 5*C* (http://www.jneurosci.org/cgi/content/full/26/10/2635/F5)]. These data certainly represent the most important findings of this study.

In conclusion, Vendel et al. (2006a) provide evidence that alternative splicing of the N-terminal A domain of the $Ca_v\beta_4$ auxiliary subunit confers functions other than modulations of channel gating and trafficking. Because $Ca_v\beta_{4a}$ splicing variant can bind synaptotagmin I, an important protein for presynaptic vesicle release, the β subunit, could conceivably act as a scaffolding element to facilitate coupling of calcium signaling with neurotransmitter release (Fig. 1). However, the authors do not provide evidence that $Ca_v\beta_{4a}$ can bind $Ca_v2.1$ channel and Syt I simultaneously. Pull-down experiments performed by preincubating the full-length $Ca_v\beta_{4a}$ with the AID peptide (the molecular determinant of the $Ca_v2.x$ channel, which interacts with the AID-binding pocket of the $Ca_v\beta$ subunit) before adding Syt I could partially answer this question. Finally, the fact that $Ca_v\beta_{4a}$–Syt I interaction is disrupted by Ca^{2+} is interesting. In this context, we speculate that at basal Ca^{2+} levels, $Ca_v\beta_{4a}$ interacts both with $Ca_v2.1$ and Syt I to organize the vesicle-release machinery and that calcium entry into cells via voltage-gated calcium channels breaks this interaction, thus releasing the vesicle to allow fusion with the plasma membrane. FRET experiments using tagged Syt I and $Ca_v\beta_{4a}$ in the presence of $Ca_v2.1$ channels before and during membrane depolarization might address such a possible mechanism.

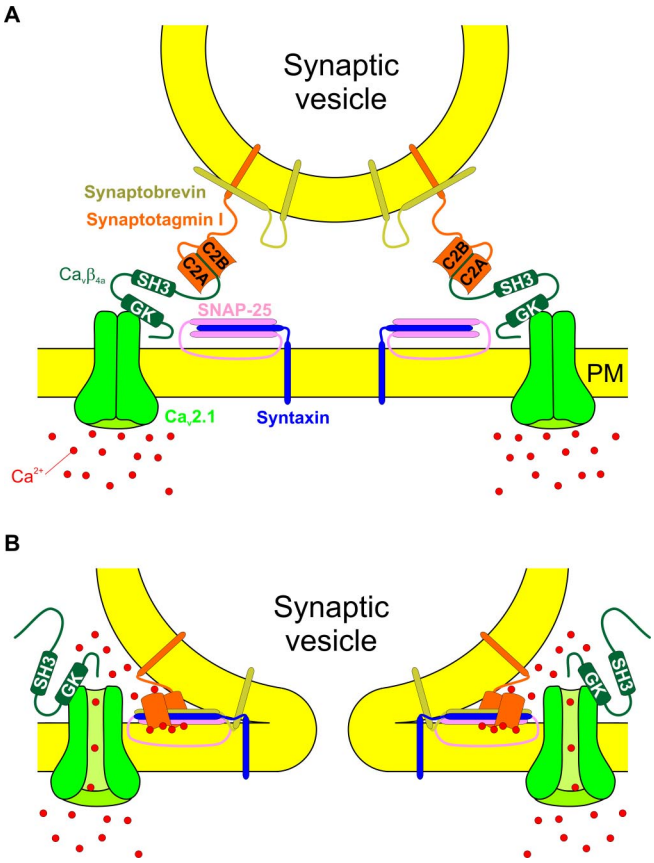

Figure 1. A putative model of coupling between voltage-gated calcium channels, calcium signaling, and presynaptic vesicle-release machinery. ***A***, In basal condition with a low Ca^{2+} level, $Ca_v\beta_{4a}$ interacts both with $Ca_v2.1$ channel and Syt I to position the synaptic vesicle in front of the channel to better couple calcium signaling and neurotransmitter release. ***B***, Calcium entry into the cell via voltage-gated calcium channels and after membrane depolarization disrupts $Ca_v\beta_{4a}$–Syt I interaction. The content of the synaptic vesicle is thus released by the fusion of the vesicle with the plasma membrane after soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors v-SNAREs (synaptobrevin and synaptotagmin I) and t-SNAREs (syntaxin and SNAP 25) interactions. PM, Plasma membrane.

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