SUPPLEMENTAL DATA

Supplemental data on the investigation of potential interacting partners with the N-terminus of $Ca_V2.2$ using the yeast two hybrid assay

Methods

Assays were carried out using the MATCHMAKER GAL4 yeast two hybrid kit (Clontech). Fragments of $Ca_V2.2$ within the first domain (amino acids 96-355, 180-225, 246-330), within the $Ca_V2.2$ N-terminus (1-95, 45-95, 56-95, 45-55), the $Ca_V2.2$ I-II loop (360-483), II-III loop (711-1154), III-IV loop (1422-1476), C-terminus (1713-2339) and $Ca_V\beta1b$ were generated by PCR and subcloned in-frame into the vectors pACT2 and pAS2-1. Plasmids were cotransformed into the yeast strain Y190 and transformants were selected by plating onto minimal selective dropout (SD) *-Leu, -Trp* agar. Protein interactions were identified by re-streaking colonies onto SD *-Leu, -Trp* plates and carrying out colony-lift β-galactosidase assays according to the supplied protocol.

Results

We were unable to demonstrate any positive interactions between the N-terminus of $Ca_V2.2$ and the $Ca_V2.2$ I-II loop or $Ca_V2.2$ Dom I using the yeast two hybrid assay, in contrast to a previous study (1). The $Ca_V2.2$ N-terminus was also truncated into smaller fragments (amino acids 45-95, 56-95 and 45-55), none of which were shown to interact with $Ca_V2.2$ I-II linker. We also made fusion proteins containing regions of domain I of the $Ca_V2.2$; the transmembrane domains (amino acids 96-355), the Domain IS4 trans-membrane region (180-225) and the p-loop region (246-330). None of these were found to interact with the full-length (1-95) or the truncated (45-95) N-terminus of $Ca_V2.2$ in this assay. In addition, dimerization was investigated for each insert and found to be negative for all except $Ca_v\beta1b$. All fragments were tested and found negative for autoactivation. As a positive control in all assays, the $Ca_V2.2$ I-II loop was shown to interact with $Ca_V\beta1b$, as described previously (2). Therefore we cannot identify a high affinity interaction of the $Ca_V2.2$ N-terminus with a particular peptide domain in the yeast two hybrid system, providing some support for the view that the N-terminus is more likely to interact in a complex binding pocket, involving multiple elements (Table 1).

Table 1

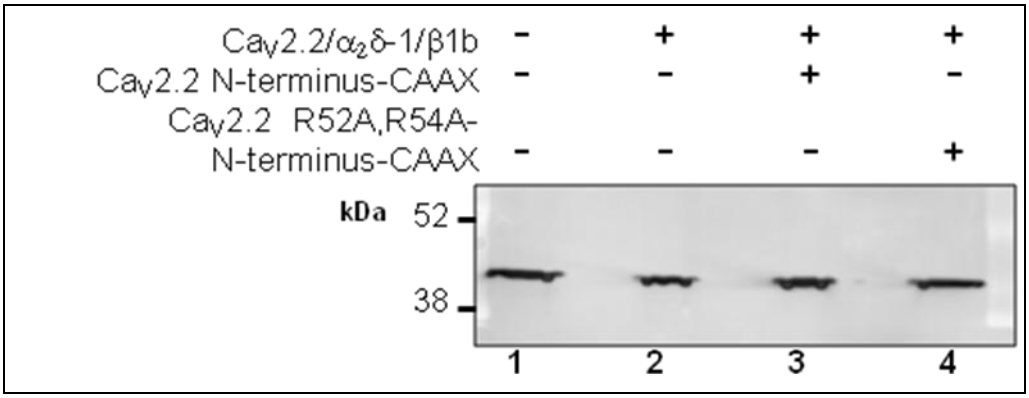
Summary of potential interactions examined by yeast two hybrid

	Ca_V2.2 N-term (1-95)	**Ca_V2.2 N-term (45-95)**	**Ca_V2.2 N-term (56-95)**	**Ca_V2.2 N-term (45-55)**	**Ca_vβ1b**
Ca_V2.2 N-term (1-95)	-	ND	ND	ND	-
Ca_V2.2 I-II (360-483)	-	-	-	-	+++
Ca_V2.2 II-III (711-1154)	-	-	ND	ND	-
Ca_V2.2 III-IV (1422-1476)	-	ND	ND	ND	-
Ca_V2.2 C-term (1713-2339)	-	-	-	-	-
Ca_V2.2 Dom I (96-355)	-	-	ND	ND	-
Ca_V2.2 Is4 (180-225)	-	-	ND	ND	ND
Ca_V2.2 I-p-loop (246-330)	-	-	ND	ND	ND
Ca_vβ1b	-	-	ND	ND	+

Assays were carried out using the MATCHMAKER GAL4 yeast two hybrid kit (Clontech). Fragments of Ca_V2.2 within the first domain (amino acids 96-355, 180-225, 246-330), within the Ca_V2.2 N-terminus (1-95, 45-95, 56-95, 45-55), the Ca_V2.2 I-II loop (360-483), II-III loop (711-1154), III-IV loop (1422-1476), C-terminus (1713-2339) and Ca_Vβ1b were generated by PCR and subcloned in-frame into both vectors pACT2 and pAS2-1. Each combination of vectors was tested for each insert. Protein interactions were identified by re-streaking colonies onto SD *-Leu, -Trp* plates and carrying out colony-lift β-galactosidase assays according to the supplied protocol. For each combination, at least 8 colonies were tested, except for reactions involving Ca_V2.2 II-III and III-IV where 2-4 colonies were tested. Interactions are indicated as +++ strong interaction (β-galactosidase assay develops in 30-60 min), + weaker interaction (1-6h), - negative interaction, ND: not determined.

Supplemental Figure 1

Expression of GAPDH as a loading control



Expression of GAPDH protein in untransfected tsA-201 cells (lane 1), when $Ca_V2.2/\alpha_2\delta$-1/β1b were expressed, alone (lane 2) and together with $Ca_V2.2$ N-terminus-CAAX (lane 3), or $Ca_V2.2$ R52A, R54A-N-terminus-CAAX (lane 4). The same amount of total protein was loaded for all samples on a gel, for accurate comparison between lanes. These samples were the same as those illustrated in Fig. 6*A*. The primary Ab was a mouse anti-GAPDH monoclonal at 1:25,000. This experiment was replicated 3 times for other samples used for the bar chart in Fig. 6*B*, with identical results that GAPDH was unchanged.

References

1. Agler, H. L., Evans, J., Tay, L. H., Anderson, M. J., Colecraft, H. M., and Yue, D. T. (2005) *Neuron* **46,** 891-904

2. Dresviannikov, A. V., Page, K. M., Leroy, J., Pratt, W. S., and Dolphin, A. C. (2009) *Pflugers Arch.* **457,** 743-756