The function of external domain in the first repeat of the $Ca_v3.1$ channel

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A sequence comparison between low and high voltage activated calcium channels revealed an extremely large external domain within the first repeat of all Ca_v3 subunits located between the IS5 transmembrane segment and the pore loop (P), which encompasses nearly 100 residues. The function of this domain containing 6 conserved cysteine residues is unknown. The aim of our study was to analyze role of cysteines in external IS5-P linker of the $Ca_v3.1$ channel. Each of cysteines was exchanged for an alanine. Resulting mutants (C241A, C271A, C282A, C323A, C298A and C313A) were transfected into HEK 293 cells. Whole-cell patch-clamp was used for current analysis. Four of channel mutants did not form functional channels: C241A, C271A, C282A and C323A, possibly because of incorrect protein folding. Two remaining channels C298A and C313A were capable of carrying inward calcium current. Application of reducing agent DTT altered current amplitude: $Ca_v3.1$: 77 ± 4 %, C298A: 40 ± 4 %, C313A: 115 ± 2 %. DTNB (Ellman's reagent) inhibited current through all three channels: $Ca_v3.1$: 1 ± 1 %, C298A: < 1 %, C313A: 48 ± 4 %. All values refer to current amplitude in presence of each reagent expressed as percentage of current amplitude measured under the control conditions.

We concluded that all cysteines in IS5-P linker of the $Ca_v3.1$ channel are essential for channel function. However, nature of their contribution is different.

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