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Title: Structure-activity studies on the opening- and closure-mechanism of L-type calcium-channels.

Abstract in Englisch

The objective of the present study was to identify gating sensitive positions in the segments of the cardiac L-type Cav1.2 channel. This was supported by homology models that suggested structural details of certain amino acid regions. The main part of this thesis focuses on the highly conserved "G/A/G/A" motif of the bundle crossing region in the S6 segments. A well known autosomal dominant point mutation which causes a channelopathy called Timothy Syndrome is located inside this motif. This inherited disease leads to arrhythmias, autism and neurological defects. The source of this channelopathy is mutation G423S which dramatically reduces inactivation. The prolonged opening of the channel causes increased calcium influx. This work shows that mutations in all analogous positions in domains II, III and IV accelerate activation. Inactivation in Domains II-IV is not affected. Since inactivation is Domain I specific, a decoupling of the inactivation and the deactivation process can be shown. Homology modelling demonstrates that all amino acids of the "G/A/G/A" motif form a tightly packed ring in the closed conformation of the channel.

We hypothesize that mutating this ring prevents complete channel closure. To investigate influence of voltage sensors on the identified "G/A/G/A" motif, all charged positions in each of the S4 segments were neutralized. Neutralization of the S4 segments in domains I, III and IV lead to a non-functional channel. Interestingly the activation shift of the neutralized segment in domain II (IIS4N) could not be distinguished from the wild-type channel. Combination of IIS4N with all "G/A/G/A" mutants caused a rescue behaviour with wild-type like activation. In contrast IIS4N in conjunction with amino acid mutations next to the "G/A/G/A" motif showed no rescue behaviour. This indicates the important function of the "G/A/G/A" motif. Thermodynamic analysis showed that the voltage sensor IIS4 is energetically coupled to the "G/A/G/A" ring. This coupling leads to conformational changes of domains II, II and IV.