Calmodulin kinase II is involved in voltage-dependent facilitation of the L-type Cav1.2 calcium channel: Identification of the phosphorylation sites.

Abstract:
Calcium-dependent facilitation of L-type calcium channels has been reported to depend on the function of calmodulin kinase II. In contrast, the mechanism for voltage-dependent facilitation is not clear. In HEK 293 cells expressing Ca(v)1.2, Ca(v)beta2a, and calmodulin kinase II, the calcium current measured at +30 mV was facilitated up to 1.5-fold by a 200-ms-long prepulse to +160 mV. This voltage-dependent facilitation was prevented by the calmodulin kinase II inhibitors KN93 and the autocamtide-2-related peptide. In cells expressing the Ca(v)1.2 mutation I1649E, a residue critical for the binding of Ca2+-bound calmodulin, facilitation was also abolished. Calmodulin kinase II was coimmunoprecipitated with the Ca(v)1.2 channel from murine heart and HEK 293 cells expressing Ca(v)1.2 and calmodulinkinase II. The precipitated Ca(v)1.2 channel was phosphorylated in the presence of calmodulin and Ca2+. Fifteen putative calmodulin kinase II phosphorylation sites were identified mostly in the carboxyl-terminal tail of Ca(v)1.2. Neither truncation at amino acid 1728 nor changing the II-III loop serines 808 and 888 to alanines affected facilitation of the calcium current. In contrast, facilitation was decreased by the single mutations S1512A and S1570A and abolished by the double mutation S1512A/S1570A. These
serines flank the carboxyl-terminal EF-hand motif. Immunoprecipitation of calmodulin kinase II with the Ca(v)1.2 channel was not affected by the mutation S1512A/S1570A. The phosphorylation of the Ca(v)1.2 protein was strongly decreased in the S1512A/S1570A double mutant. These results suggest that voltage-dependent facilitation of the Ca(v)1.2 channel depends on the phosphorylation of Ser1512/Ser1570 by calmodulin kinase II.