Identification of a site involved in the block by extracellular Mg^{2+} and Ba^{2+} as well as permeation of K^+ in Kir2.1 K^+ channel

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Summary

The inward rectifier potassium channel Kir2.1 is more sensitive to the weakly voltage-dependent block by extracellular Mg^{2+} (Mg^{2+}_o) than Kir2.2 and Kir2.3. We identified Glu125 at an extracellular loop before the pore region of Kir2.1 as a responsible site for its sensitivity to Mg^{2+}_o block, since Glu125Gln (E125Q) mutation strongly decreased the sensitivity while a mutation to Glu at corresponding sites of Kir2.2 and 2.3 increased it. The negative charge was proved to be crucial because Glu125Asp (E125D) mutant showed properties similar to the wild type (wt). The sensitivity to the block by extracellular Ba^{2+} (Ba^{2+}_o) was also decreased in E125Q mutant although the depth of the block was not changed, as reported previously. We additionally observed that the speed of Ba^{2+}_o block and recovery was decelerated by the presence of Mg^{2+}_o in wt but not in E125Q mutant. The sensitivity to the block by Mg^{2+}_o was increased by lowering extracellular K^+ (K^+_o), suggesting a competitive interaction of Mg^{2+}_o and K^+_o. The single channel conductance of wt in 140 mM K^+ was 39.6 pS (0 mM Mg^{2+}_o) and 11.5 pS (10 mM), while that of E125Q mutant was 26.0 pS (0 mM) and 19.6 pS (10 mM). These results demonstrate that Mg^{2+} competes with K^+ permeation in wt and that E125 is requisite for efficient K^+ permeation in the absence of Mg^{2+}_o. Taken together with these results, we conclude that E125 at an extracellular loop of Kir2.1 is an intermediate binding site which facilitates K^+ permeation and entry of Ba^{2+} toward a deeper plugging site, and that Mg^{2+}_o competes with K^+_o and Ba^{2+}_o at this site.