Structure-Activity study of the Transient Receptor Potential Canonical (TRPC6) Channels

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Introduction: Transient receptor potential canonical (TRPC6) channels play an important role in Ca^{2+} entry into several physiological and pathophysiological processes. Mutations in TRPC6 gene lead to focal segmental glomerulosclerosis, but their pathogenic mechanism remains elusive. TRPC6 gating modulation mechanism is still unknown. Recent results from our laboratory shown that Ca^{2+} and Mg^{2+} influence the structure of the C-terminal's helicoidal domain of TRPC6. These interactions are localized in the cytosolic part of the channel and may have an essential role in the regulation of TRPC6.

Aims: To determine the binding site of Ca^{2+} and its role in the activation / regulation of TRPC6.

Methods: By using the site-directed mutagenesis strategy, different mutants were generated, in the linker between the horizontal helical domain and the vertical helical domain, where aspartic acid residues were substituted by asparagine, glutamic acid residues were substituted by glutamine, and each of them were substituted by alanine, they were expressed in HEK293 cell. Then lipofection protocol was be optimized to make stably transfected with the Gαq-coupled M5 muscarinic receptor in HEK293 cells. Then, the $[Ca^{2+}]_i$ was measured into the cells by Ca^{2+} imaging in real time using a fura-2 Ca^{2+} probe. Then, the Ca^{2+} entry was evaluated using the Ca^{2+} depletion/re-addition protocol in fura-2 loaded cells after stimulation with carbachol (CCh), a muscarinic receptor agonist.

Results: Ca^{2+} depletion was induced with 10 µM CCh in the Ca^{2+} free (0.5 mM EGTA) HBSS solution. Once the $[Ca^{2+}]_i$ had returned to the basal level (3 min after the addition of CCh), the extracellular medium was repleted with 1.8 mm $CaCl_2$. In the absence of extracellular Ca^{2+}, CCh-induced Ca^{2+} release was similar whether cells were transfected with $TRPC6^{WT}$ or mutants TRPC6. After adding 1.8 mm $CaCl_2$ to the external medium, $TRPC6^{Wt}$, $TRPC6^{E878Q}$, and $TRPC6^{D877N}$ demonstrated considerably higher intracellular calcium concentrations than the control group, according to a one-way ANOVA statical test ($p<0.0001$). Dunnett's tests showed significant increases in $[Ca^{2+}]_i$ between the control group and $TRPC6^{Wt}$ ($p <0.0001$) and between $TRPC6^{Wt}$ and $TRPC6^{D877N}$ ($p= 0.0327$) after multiple comparisons. When 1.8 mm CaCl2 was added, $TRPC6^{E878Q}$ intracellular calcium concentration changed somewhat from $TRPC6^{Wt}$, but not insignificantly ($p= 0.3468$). **B**: The change in the level of intracellular calcium concentration after adding 1.8 mm CaCl2 in TRPC6Wt compared to the control group was insignificant ($p< 0.0086$), while between the TRPC6E878A and TRPC6D877A compared to TRPC6Wt was not insignificant($p> 0.05$). Quantitative protein expression in HEK 293 cells confirmed the results in Ca^{2+} imagine as well.

Conclusion: Based on the results, we identified calcium binding sites in TRPC6 and confirm these acidic residues contribute to forming a binding pocket for Ca^{2+}.