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MEETING ABSTRACT

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Phosphorylation of $K_V7.2$ regulates its PIP_2 sensitivity

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Background: K_V7 channels are a subfamily of voltage-gated K^+ channels that play a major role in the regulation of neuronal excitability. G_q-coupled receptors like the M_1 acetylcholine receptor can regulate K_V7 channel function by affecting the levels of PIP_2, which is required for channel opening. On the other hand, phosphorylation is also involved but an interaction of these pathways has not been well explored.

Methods: We used liquid chromatography–mass spectrometry to identify phosphorylation sites from rat brain and transfected heterologous cells. To evaluate the effect of phosphorylation on PIP_2-mediated $K_V7.2$ regulation, we generated the dephosphomimetic A^5 mutant (S427/436/438/446/455A^5) and reduced the PIP_2 levels by activating the voltage-sensitive phosphatase Dr-VSP. *In vitro* phosphorylation assays were performed to determine the responsible kinases phosphorylating the sites in the PIP_2-binding domain. Cells were treated with a mixture of the respective kinase inhibitors (roscovitine, SB 203580, KN-62, H-7). Rat primary neurons of the superior cervical ganglia (SCG) were cultured and treated with the inhibitor mix before evaluating the effect of PIP_2 depletion by activating the M_1 receptor with increasing concentrations of oxotremorine methiodide (Oxo-M).

Results: We identified 13 phosphorylation sites in immunopurified $K_V7.2$. Among them, five phosphorylation sites were clustered in one of the putative PIP_2-binding domains. Dr-VSP needed longer to inhibit the current in the dephosphomimetic mutant compared to wild-type $K_V7.2$. CDK5, p38 MAPK, CaMK II and PKA were found *in vitro* to phosphorylate the identified sites in the PIP_2-binding domain. Thus, we inhibited the kinases with the respective inhibitors and observed a similar Dr-VSP response as with the dephosphomimicking mutant. Furthermore, pretreating SCG neurons with the inhibitor mix and depleting PIP_2 by using increasing concentrations of Oxo-M resulted in a similar decrease of M current inhibition.

Discussion: Our results suggest that phosphorylation of $K_V7.2$ in the putative PIP_2-binding domain determines its PIP_2 sensitivity.

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