Salzer et al. BMC Pharmacology and Toxicology 2012, 13(Suppl 1):A46
http://www.biomedcentral.com/2050-6511/13/S1/A46

BMC Pharmacology & Toxicology

MEETING ABSTRACT Open Access

Functional implications of K_V7 channel phosphorylation

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From 18th Scientific Symposium of the Austrian Pharmacological Society (APHAR). Joint meeting with the Croatian, Serbian and Slovenian Pharmacological Societies.
Graz, Austria. 20-21 September 2012

Background

The family of K_V7 potassium channels, particularly $K_V7.2$, $K_V7.3$, and $K_V7.5$ controls neuronal excitability. Numerous neurotransmitters acting via G protein-coupled receptors signaling via Ca^{2+}/calmodulin or depletion of membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) tightly regulate K_V7 channel function. Moreover, the phosphorylation of K_V7 channels has been proposed to play a crucial role. However, *in vivo* phosphorylation sites and their functional implications need to be determined.

Methods

To investigate the role of steady-state K_V7 channel phosphorylation, superior cervical ganglion (SCG) neurons were pretreated for 30 min with different kinase inhibitors (GW8510: 10 μM, SB415286: 1 μM, SB203580: 10 μM, H7: 10 μM) which block CDK5, GSK3, p38 MAPK, and PKC as well as PKA, respectively. Thereafter, oxotremorine M (OxoM) or bradykinin-induced inhibition of the M-currents (primarily through $K_V7.2/7.3$ heterotetramers) was tested.

Results

Inhibition of CDK5 shifted the concentration-response curve for OxoM to the left, but not that of bradykinin. Similarly, GW8510 treatment of tsA201 cells, heterologously expressing $K_V7.2$ channels and M_1 receptors, caused a leftward shift of the OxoM concentration-response relation. In mass-spectrometric studies, several phosphorylated amino acid residues in the C-terminus of native and heterologously expressed $K_V7.2$ channels were detected, 5 of them are located within the putative PIP_2 binding site. CDK5 was predicted to target serines S427 and S446. In contrast to S446, mutation of S427 to alanine significantly increased $K_V7.2$ channel sensitivity towards inhibition via M_1 receptors. Additionally, treatment with GW8510 failed to cause any further effect. Nevertheless, these alanine mutations did not influence the channel-voltage dependence.

Conclusions

Hence, phosphorylation of C-terminal serine residue 427 determines $K_V7.2$ modulation by M_1 muscarinic, but not by B_2 bradykinin receptors, suggesting that the phosphorylation state of S427 regulates the affinity of the $K_V7.2$ C-terminus for PIP_2.

Acknowledgements
The study is supported by the Austrian Science Fund (grants P23670-B09 and P19710), and the PhD programme CCHD of the Medical University of Vienna.

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Published: 17 September 2012

doi:10.1186/2050-6511-13-S1-A46
Cite this article as: Salzer *et al.*: Functional implications of K_V7 channel phosphorylation. *BMC Pharmacology and Toxicology* 2012 **13**(Suppl 1):A46.

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