Proposal for a presentation for the Belgian Society for Fundamental and Clinical Physiology and Pharmacology

Interactions of apamin with pore mutated SK3 channels.

S. Dilly[1,2], C. Lamy[2], S. Poncin[2], V. Seutin[2], D. Snyders[3] and J.-F. Liégeois[1]

[1]Laboratory of Medicinal Chemistry and C.I.R.M., University of Liège, B-4000 Liège 1, Belgium [2]Laboratory of Pharmacology and GIGA-Neurosciences, University of Liège, B-4000 Liège 1, Belgium [3]Laboratory for Molecular Biophysics, Physiology and Pharmacology, Department of Biomedical Sciences, University of Antwerp, B-2610 Antwerp, Belgium.

In the present work, we have tested the impact of the replacement of valine residues in the pore region of SK3 (520) by either an alanine or a phenylalanine residue in terms of the interactions of apamin with these mutants in comparison with the corresponding native channels. Replacing valine residue at position 520 of the SK3 channel by a phenylalanine significantly increased the sensitivity of the channel to be blocked by tetraethylammonium (TEA) as previously reported. Indeed, an aromatic residue, such as a phenylalanine or a tyrosine, is frequently found in the pore region of several potassium channels more sensitive to TEA than SK channels.

We measured the affinity (K_d) of apamin in saturation experiments and studied SK currents in transfected cells using patch clamp techniques.

In parallel, molecular modelling techniques were used to examine the impact of these local modifications on the interaction of apamin with the corresponding channels. The presence of a phenylalanine in the pore region of potassium channels led to a higher sensitivity for TEA by creating more hydrophobic interactions as found by the docking procedure. In the *in vitro* binding experiments, the phenylalanine mutant (SK3VF) displayed a very low affinity for apamin. In patch clamp experiments, the SK current was only very partially blocked by apamin in the SK3VF mutant. Furthermore, apamin displayed an affinity and a blocking activity for the alanine mutant close to that for the corresponding native channels. In conclusion, the presence of a bulky and hydrophobic residue at a position near the pore mouth of SK3 channels has a negative impact on their interactions with apamin.

I want to present this presentation as poster