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Abstract: 96O

A fluorescently-tagged peptide toxin, Cy5-HsTX1[R14A], as a tool for K_V1.3 visualisation

Dorothy C.C. Wai[a], Muhammad Umair Naseem[b], Sanjeevini Babu Reddiar[a], Yijun Pan[a], Gabor Mocsár[b], Ágota Csóti[b], Gyorgy Panyi[b], Joseph A. Nicolazzo[a], Raymond S. Norton[a]

[a] *Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3052, Australia.*
[b] *Department of Biophysics and Cell Biology, University of Debrecen, 4032 Debrecen, Hungary.*

The voltage-gated potassium channel K_V1.3 plays an important role in the activation of T cells and microglia [1,2]. Homotetrameric K_V1.3 channels are specifically upregulated in effector memory T-cells, which have been implicated in autoimmune diseases including rheumatoid arthritis, psoriasis, multiple sclerosis and type I diabetes [1]. Microglia, which also express K_V1.5, upregulate K_V1.3 in neuroinflammatory diseases such as Alzheimer's and Parkinson's disease [6]. It is of interest to be able to specifically identify and visualise homotetrameric K_V1.3 channels, which have distinct functional properties from other K_V1 channels and K_V1.3 heterotetramers [5]. Antibodies or small molecules are unable to distinguish homotetrameric K_V1.3 channels from heterotetrameric K_V1.3/K_V1.x channels. In contrast, a number of animal-derived peptide toxins that bind to the extracellular vestibule of the channel exhibit selectivity for K_V1.3 homotetramers [3]. HsTX1[R14A] is an analogue of a 34-residue peptide toxin from the scorpion *Heterometrus spinifer* that binds K_V1.3 with an IC_{50} of 45 pM and displays a 2000-fold selectivity for K_V1.3 over K_V1.1 [4]. We have synthesised a fluorescent analogue of HsTX1[R14A] by N-terminal conjugation of a Cy5 tag. Electrophysiology assays show that Cy5-HsTX1[R14A] retains nanomolar activity against K_V1.3 and selectivity over a range of other potassium channels as well as heteromeric K_V1.3/K_V1.5 channels. Live-cell imaging of CHO cells expressing GFP-K_V1.3 shows colocalisation of Cy5-HsTX1[R14A] and K_V1.3 fluorescence signals at the cell membrane. Cy5-HsTX1[R14A] is also able to detect K_V1.3 at physiologically relevant expression levels in lipopolysaccharide-stimulated mouse microglia. Furthermore, the tissue-penetrating far-red emission profile of Cy5 affords the potential to visualise the biodistribution of the peptide and K_V1.3 *in vivo*, as illustrated by our preliminary studies in healthy mice. These results highlight the utility of Cy5-HsTX1[R14A] as a K_V1.3 probe, which will have broad applicability in fundamental investigations of K_V1.3 trafficking and K_V1 channel composition, as well as in validation of novel disease indications where K_V1.3 inhibition may be of therapeutic value.

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