Analysis of large conductance Ca^{2+}-activated K^+ (BK) channels dynamics in living cells

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KEY WORDS: BK channels, diabetes, secretory vesicles, secretory machinery, super-resolution microscopy, electrophysiology.

Large conductance Ca^{2+}-activated K^+ (BK) channels are expressed in a wide range of human tissues such as brain, thymus, muscle, adrenal gland, heart, lung or pancreas, where they regulate insulin secretion by closing N-type calcium channels [1]. The study of BK channels activity, localization and dynamics, would be useful not only for the achievement of a further understanding of BK channels properties in physiological conditions, but also in disease. Indeed, BK channel activity has been reported to be disrupted in a wide range of disorders, such as diabetes [2]. Therefore, our study aims to determine whether components of the secretory machinery and BK-channels are localised in close proximity at the plasma membrane, as well as how the secretory machinery influences both BK channel localisation and activity. To carry out these experiments we are using super-resolution microscopy and electrophysiology.

Previous electrophysiological studies have suggested that BK channels and components of secretory machinery must be in close proximity in the plasma membrane [3]. Therefore, we observed the dynamics and distribution of BK channels at the level of single molecules in cell membranes using super-resolution microscopy. We observed BK channel cluster organization of 200-300nm in diameter in cells expressing BKα-EGFP using gated-stimulated emission depletion (g-STED) microscopy. Moreover, to further examine these clusters we expressed a cDNA construct that encodes the BK-channel α-subunit (BKα) fused to a photoactivatable fluorescent protein mutant of mCherry (PAmCherry) and localised single channel molecules using photoactivatable localisation microscopy (PALM), which can localise where each single particle is within each cluster, and the distance respect to their closest secretory vesicles using Nearest Neighbour analysis. The results using Bayesian analysis showed that about 30-40% of BK channels are organised in clusters. Further experiments in live cells using single particle tracking PALM (sptPALM) enabled us to track these single molecules over time showing different speeds of BK channels with and without the presence of the secretory machinery components. Additionally, to study the proximity of BK channels with the secretory machinery cells were transfected with our BKα-EGFP construct and Syntaxin-1A-mCherry, a plasma membrane protein involved in vesicle fusion, and analysed using Fluorescence Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging (FLIM). Results suggested that both BKα and Syntaxin-1A interact in the plasma membrane. On the other hand, the electrophysiological experiments with cells co-expressing both BK channels and Syntaxin-1A suggest a non-statistically significant decrease of BK channels conductance in the presence of this secretory machinery component [4].

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[4] The author gratefully acknowledges funding from Medical Research Council (MRC) and Edinburgh Super-resolution Imaging Consortium (ESRIC).