

## Screening the effects of cysteine mutations on BK<sub>Ca</sub> channel function and trafficking

Nina Geiger, Owen Jeffries, Fozia Saleem, Iain Rowe, Michael Shipston. University of Edinburgh, Edinburgh, United Kingdom.

Large conductance voltage and calcium-activated potassium (BK<sub>Ca</sub>) channels link electrical excitability with cellular signalling. A single gene (*KCNMA1* or *slo*) encodes all four  $\alpha$ -subunits that shape the native BK<sub>Ca</sub> channel pore. Alternative splicing of the mRNA determines the channel phenotype, including its intrinsic voltage dependence and calcium sensitivity (Chen *et al.*, 2005). The identification and characterization of BK<sub>Ca</sub> channel splice variants and the functional impact of point mutations within regulatory motifs in the channel sequence normally depends on the use of low throughput patch clamp electrophysiology. Previously we have developed a robust, high throughput functional assay to screen BK<sub>Ca</sub> splice variants using FLIPR (fluorescent-imaging plate reader) Membrane Potential (FMP) dyes (Saleem *et al.*, 2009). We have now combined this technique with imaging of epitope-tagged channel constructs to determine cell surface expression in order to assess the impact of cysteine point mutations on channel function and trafficking.

Ionomycin-induced hyperpolarisation of HEK293 cells expressing: the BK<sub>Ca</sub> channel ZERO variant (without splice inserts); the stress-regulated exon variant (STREX); and constructs with cysteine point mutations; were measured and expressed as a percentage of the STREX channels response. ZERO, STREX C12A, C13A and C12:13A produced significantly smaller responses, of  $18 \pm 22\%^{***}$ ,  $5 \pm 3\%^{***}$ ,  $57 \pm 9\%^{*}$  and  $74 \pm 4\%^{*}$  respectively, when normalised to the STREX response (taken as 100%) (N = 3, n = 12, data are mean  $\pm$  sem, \* p<0.05 & \*\*\* p<0.001 ANOVA with Tukey post-hoc test). There were no significant differences in the levels of surface expression between these channel variants and mutants.

In conclusion, the combination of voltage-sensitive dyes and epitope-tagged channel constructs can be used to generate reliable, reproducible data on the functional impact of cysteine mutations on BK<sub>Ca</sub> channel activity and trafficking.

Chen et al. (2005) *J Biol Chem* **280**, 33599-33609.

Saleem et al. (2009) *Br J Pharmacol* **156**, 143-152.

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