EXPRESSION AND CHARACTERISATION OF A TWO PORE POTASSIUM CHANNEL IN HEK293 CELLS USING DIFFERENT ASSAY PLATFORMS.

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Recent discovery, through molecular cloning, has revealed a family of K^+ channels with properties distinct from previously identified K^+ channels families. This family has been termed the two-pore domain K^+ channel family (KCNK) and their leak K^+ conductances are believed to be important in setting neuronal membrane potential and regulation of cellular excitability (Bayliss *et al*., 2003). One particular subgroup of the KCNK family or TASK channels are inhibited by acidic extracellular pH and modulated by some local and volatile anaesthetics. The properties and distribution of the TASK channels makes them an interesting drug discovery target. We have produced a HEK293 cell line stably expressing the TASK-3 K^+ channel and characterised the cell line using three different assay platforms, atomic emission spectroscopy (Rb^+ efflux), automated electrophysiology (IonWorks® Quattro™) and conventional electrophysiology.

Human TASK3 was isolated from a brain cDNA library and cloned into a bicistronic expression vector containing the CD8 selection marker. A stable HEK293 cell line expressing TASK3 was isolated by dilution cloning and screened for functional expression using Rb^+ efflux, IonWorks Quattro and conventional patch-clamp techniques.

A voltage ramp protocol (-80 to +60mV, 500ms) was used to elicit currents from a holding potential of -80mV using the IonWorks Quattro and conventional patch-clamp. The mean current amplitude (±sem) at +60mV was 1.42±0.14nA (*n*=30 cells) and 2.58±0.47nA (*n*=5 cells) using the IonWorks Quattro and convention patch-clamp techniques, respectively. The mean current amplitude at +60mV of the wild type HEK293 cells was 0.30±0.02nA (*n* = 40 cells) recorded using the IonWorks Quattro. Decreasing the extracellular pH from 7.4 to 6 caused a reduction of 84% in the signal produced in the Rb^+ efflux technique, with a mean percentage inhibition of current amplitude (±sem) at +60mV of 72.0±2.1% (*n*=18cells) and 83.2±2.0% (*n*=3cells) found using the IonWorks Quattro and conventional patch-clamp techniques, respectively. The Rb^+ efflux technique gave an IC_{50} for Ruthenium Red of 6.8µM and 5.8µM, with the IonWorks Quattro giving an IC_{50} of 2.5µM (*n*>3cells per concentration).

We have successfully developed a cell line that stably expresses the human two-pore domain K^+ channel, TASK3. In addition, we have shown comparative data across three different assay platforms with a range of throughputs and differing temporal resolution.

Bayliss *et al*. (2003) *Mol Interv.* **3**, 205-219.