

Using Inducible Expression Vector Technology To Create Stable Cell Lines Expressing KCNQ2/3, KCNQ4, And KCNQ3/5 Currents Suitable For Automated Electrophysiology Platforms.

Andrew P. Southan, Scott Maidment, Simon Dowler, Matthew Gardener, Anthony Lawrence, Omar Aziz, Tristana von Will, and Gary Clark.

BioFocus DPI, Chesterford Research Park, Saffron Walden, CB10 1XL (UK). email: info@biofocus.com

Introduction

The KCNQ (K_v7) family of voltage-gated ion channels conduct a number of hyperpolarising currents in various tissue types, including the heteromultimeric KCNQ2/3 M-current found in sensory neurones. Defects in KCNQ genes are responsible for a range of disorders from an inherited form of epilepsy (benign familial neonatal convulsions) to profound loss of hearing. KCNQ channels are also attractive targets for therapeutic intervention in medical conditions involving neuronal hyperexcitability such as epilepsy and neuropathic pain. Previously, stable constitutive expression of KCNQ2/3, KCNQ4, and KCNQ3/5 genes in the BioFocus DPI research laboratories produced cell lines with heterogeneous expression levels and atypical morphology that became more pronounced during routine passage. These cell lines were unsuitable for automated electrophysiology recording. We have now used inducible expression vector technology to produce stable cell lines where expression of the ion channel protein can be repressed until required for experimentation.

Materials and Methods

Cell lines

The stable cell lines created in this study were hKCNQ2/3, hKCNQ4 and hKCNQ3/5; they were maintained using standard tissue culture methods.

Electrophysiology

IonWorks® Quattro™ recordings were taken in Dulbecco's PBS using a cell density of 1.5×10^6 cells per ml (21-23 °C). The internal solution contained (in mM): NaCl 10, KGlucuronate 90, KCl 30, MgCl₂ 1, HEPES 10, EGTA 10; pH 7.38 and using 0.1 mg/ml amphotericin B to gain perforated whole-cell access. Holding potential was -80 mV. For assessing the potency of inhibitors a single 2000 ms voltage step to +40 mV was used (Figure 1A). For activator studies a 2000 ms ramp protocol from -100 mV to +100 mV was used (Figure 1B).

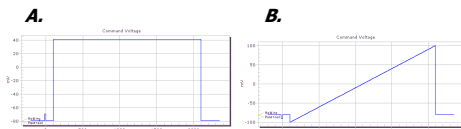


Figure 1. Voltage protocols used for assessment of both antagonist (A) and agonist (B) activity.

Results

Evaluation of cell lines using IonWorks® Quattro™

Thirty clones from each of the three KCNQ family members were tested using single-hole PatchPlates™ on the IonWorks® Quattro™. Individual clones were selected on the basis of seal resistance properties and the percentage of cells displaying suitable current expression. The selected clones were then further tested on IonWorks® Quattro™ recording in Population Patch Clamp (PPC) mode. A summary of these results is shown in Table 1.

		% wells sealed	Resistance (mean±SD)	% expression	Current (mean±SD)
KCNQ2/3	Single hole	96%	249±52 MΩ	59%	2.0±1.2 nA
	PPC	100%	159±50 MΩ	100%	1.3±0.2 nA
KCNQ4	Single Hole	97%	217±37 MΩ	77%	0.9±0.3 nA
	PPC	98%	170±62 MΩ	98%	0.6±0.1 nA
KCNQ3/5	Single hole	84%	226±63 MΩ	69%	0.8±0.3 nA
	PPC	96%	141±62 MΩ	97%	0.6±0.1 nA

Table 1. Performance of KCNQ clones assessed using IonWorks® Quattro™ in both single hole and PPC recording modes. The percentage of wells sealed was calculated from the number of wells >100 MΩ and >50 MΩ resistance for single hole and PPC experiments, respectively. The percentage expression was calculated from the number of wells showing >0.5 nA and >0.3 nA current amplitude for single hole and PPC experiments, respectively.

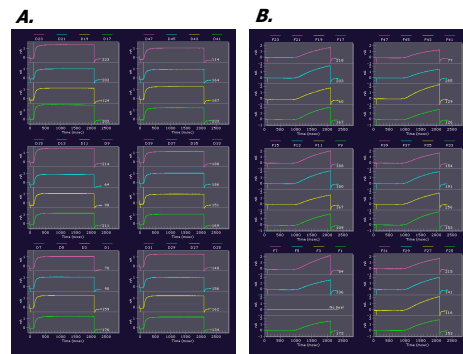


Figure 2. Example IonWorks® Quattro™ screenshots showing PPC mode recordings using the inducible KCNQ2/3 cell line. A. Outward currents observed using the voltage step protocol. B. Outward current observed using the voltage ramp protocol.

Pharmacology of standard inhibitors

The pharmacological profile of the inducible cell lines was examined using the standard KCNQ ion channel blockers TEA, linopirdine and XE991 (Table 2). An example recording from each channel type and the effects of 0.12 μM XE991 is shown in Figure 3.

Standard	KCNQ2/3	KCNQ4	KCNQ3/5
TEA	1.4 mM	40 mM	67 mM
Linopirdine	0.42 μM	41.2 μM	2.5 μM
XE991	0.07 μM	10.5 μM	0.88 μM
Retigabine	3.5 μM	n.d.	3.0 μM

Table 2. Potencies of three standard antagonists against KCNQ2/3, KCNQ4 and KCNQ3/5. Values shown for TEA, linopirdine and XE991 are IC₅₀ potencies. The EC₅₀ value for retigabine was derived from the data presented in Figure 4. All recordings performed in PPC mode using IonWorks® Quattro™.

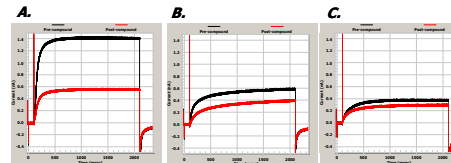


Figure 3. Example traces displaying the inhibitory effect of 0.12 μM XE991 for KCNQ2/3 (A), KCNQ3/5 (B) and KCNQ4 (C). Controls in black, XE991 in red.

Retigabine pharmacology

Activation of KCNQ currents by retigabine was measured using the ramp protocol shown in Figure 1A. Recordings were taken in the presence and absence of a range of concentrations of retigabine. Outward current was measured at appropriate time points on the ramp and conductance-voltage relationships were plotted (Figure 4). A concentration-dependent shift in the current activation was observed for both KCNQ2/3 and KCNQ3/5 with the application of retigabine. However, retigabine decreased the current at more positive potentials for KCNQ2/3, but enhanced the current for KCNQ3/5.

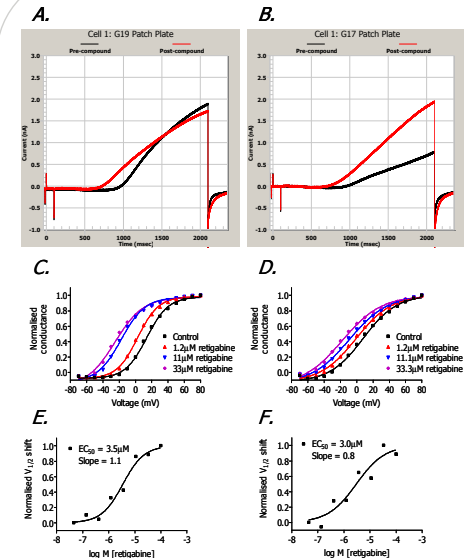


Figure 4. Example traces displaying the effect of 11.1 μM retigabine (in red) on the outward current profile of KCNQ2/3 (A) and KCNQ3/5 (B). Conductance-voltage plots in the presence and absence of retigabine, KCNQ2/3 (C) and KCNQ3/5 (D). The V_{1/2} shift plotted for KCNQ2/3 (E) and KCNQ3/5 (F).

Conclusions

- We have produced stable cell lines expressing KCNQ family ion channels without the performance limitations and morphological changes observed when using constitutive expression.
- IonWorks® Quattro™ Population Patch Clamp mode recordings have demonstrated that the cell lines give viable current amplitudes and seal resistance values for automated electrophysiology recording.
- The cell lines exhibit agonist and antagonist responses that are comparable to literature values.
- Inducible expression is an effective strategy that can be applied to ion channel targets with challenging stable expression characteristics.