OPTIMIZING CHO-hERG CELLS FOR AUTOMATED PATCH CLAMPING

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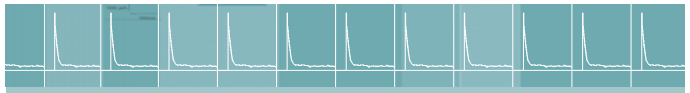

Cultured cells from mammalian cell lines incubated at 37 °C can be used for automated patch clamp (APC), including compound screening, for only a relatively brief period of time. Usually this time period extends from the time of ~50 % confluence to full confluence. For CHO cells, the preferred cell type for APC, expressing hERG potassium channels the duration of the usable period is 1-2 days. We here report that incubating CHO-hERG cells at a reduced temperature, 30 °C, for 1-5 days prior to APC experiments is advantageous in several ways: (1) it increases the percentage of cells with acceptable hERG currents (> 50 pA), (2) it increases the average whole-cell current significantly, and (3) it slows cell proliferation and extends the usable period up to at least 5 days. In addition, we have tested whether the altered conditions affect the IC_{50} obtained for known hERG inhibitors. For all experiments the automated patch clamp system QPatch 16 was used.

MATERIALS AND METHODS

Cells. Cultured CHO cells expressing hERG potassium channels were used. The cells ware incubated either at 37 °C, or initially at 37 °C and subsequently (at 70-80 % confluence) 1-5 days at 30 °C.

Ringer's solutions.
Extracellular (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH=7.4 (NaOH), ~305 mOsm. Intracellular (in mM): 5.4 $CaCl_2$, 1.75 $MgCl_2$, 10 EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP, pH=7.2 (KOH), ~290 mOsm.

Compounds. Verapamil (Sigma, Buchs, Switzerland) was applied in four concentrations from 0.03 – 30 µM in ten-fold increments. Astemizole (Sigma) was applied in four concentrations from 1.9 – 50 nM in three-fold increments.

Data analysis. Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). Drug effects were analysed as function of time (I-t plot) and concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software.

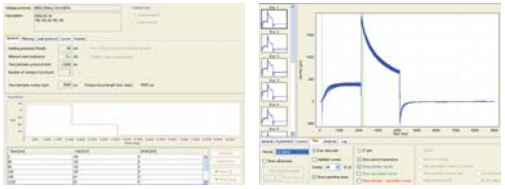

Figure 1.
Graphical representation (red graph) of the hERG voltage protocol as viewed with the QPatch assay software (left). Typical hERG whole cell currents (right). Ten single whole cell current traces superpositioned. Green cursors define time period in which the peak tail current is identified.

peak 37 °C [%]	n	incubation@ 30 °C	Peak 30 °C [%]	n
100	17	1 day	229	37
100	12	2 days	233	16
100	11	3 days	189	41
100	17	4 days	172	19
100	18	5 days	142	31

Table 1.
Incubation at 30 °C for 1-5 days increased peak tail currents relative to those obtained for cells incubated solely at 37 °C (control values, normalized to 100 %).

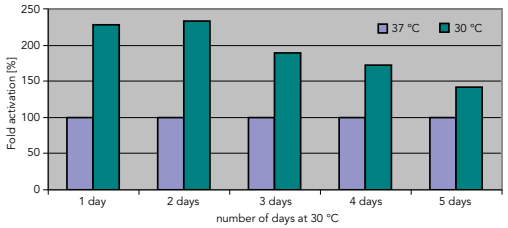

Figure 2.
Summary of normalized hERG whole-cell peak tail currents obtained either with cells cultured entirely at 37 °C (blue bars) or initially at 37 °C (until ~75 % confluence) and subsequently at 1-5 days at 30 °C (green bars).

CELL CULTURES AND hERG CURRENT TRACES BEFORE AND AFTER INCUBATION AT 30 °C

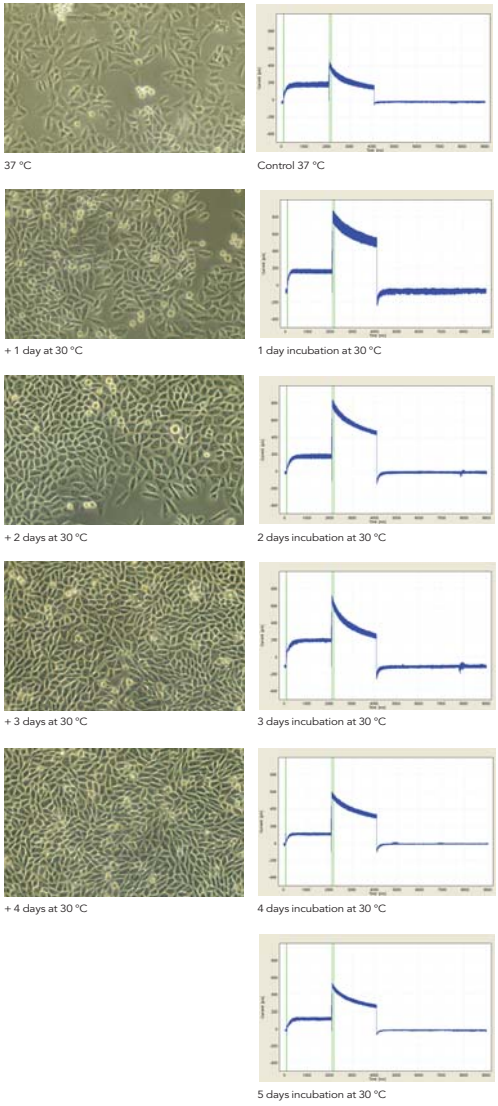

Figure 3.
CHO-hERG cultures incubated entirely at 37 °C (upper row) or initially at 37 °C and subsequently for 1-5 days at 30 °C (5 subsequent rows). Left panels show micrographs of the cell cultures. Right panels show the measured whole-cell hERG currents (10-12 traces superimposed) as viewed with the QPatch assay software.

VERAPAMIL IC_{50} DETERMINATIONS

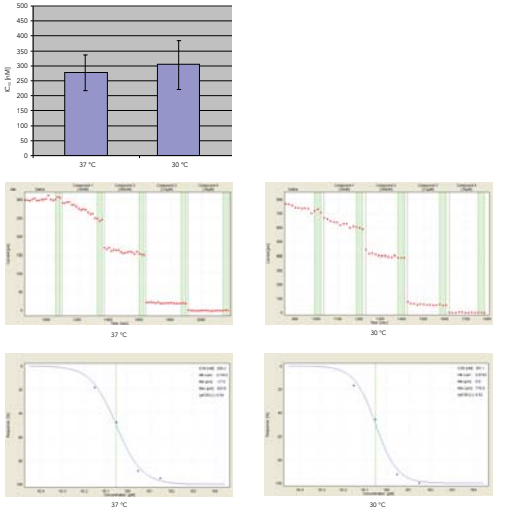

Figure 4.
Upper panel: Verapamil IC_{50} values obtained with CHO-hERG grown entirely at 37 °C (left bar, n=17), or at 37 °C until ~ 70 % confluence and subsequently for 2 days at 30 °C (right bar, n=13), were similar.
Mid panel: I-t plots showing progressive inhibition of peak tail currents with increasing Verapamil concentrations. Left panel: 37 °C (control), right panel: 2 days at 30 °C.
Lower panel: contration-response relationships for verapamil. Left panel: 37 °C, right panel: 2 days at 30 °C. Green cursors indicate IC_{50} values.

ASTEMIZOLE IC_{50} DETERMINATIONS

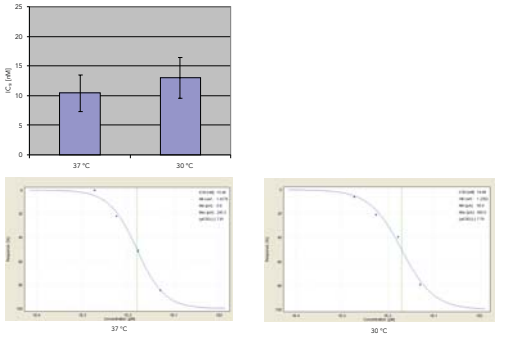

Figure 5.
Upper panel: Astemizole IC_{50} values obtained with CHO-hERG grown entirely at 37 °C (left bar, n=6), or at 37 °C until ~ 70 % confluence and subsequently for 2 days at 30 °C (right bar, n=7), were similar.
Lower panel: Astemizole contration-response relationships for Verapemil. Left panel: 37 °C, right panel: 2 days at 30 °C. Green cursors indicate IC_{50} values.

CONCLUSION

Cultured CHO cells expressing hERG potassium channels were used for APC experiments with QPatch 16. When cells were grown initially at 37 °C and subsequently 1-5 days at 30 °C, the whole-cell hERG currents were significantly higher than currents in cells cultured entirely at 37 °C. Furthermore, the time period through which the cells could be used for automated patch clamp experiments with QPatch 16 was extended from about one day to at least 5 days. Importantly, no difference in IC_{50} values was observed between the two groups. These observations demonstrate that reducing the incubation temperature from 37 to 30 °C at the time of ~70% confluence is highly advantageous for APC applications.