STRATEGIES FOR ENHANCING THROUGHPUT IN ION CHANNEL DRUG SCREENING WITH QPATCH HT

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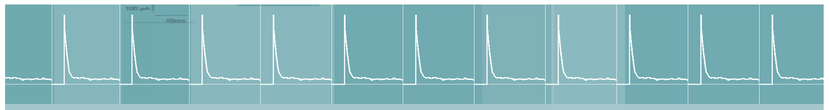


High information-content screenings based on whole-cell current patch-clamp recordings have become available for ion channel pharmacological research with the development of the QPatch automated patch-clamp technology.
A significant increase in system throughput was recently achieved by a tripling of the number of parallel operating patch-clamp sites (from 16 to 48) by the introduction of QPatch HT.
In a series of hERG screening studies we have subsequently aimed at increasing the throughput further by reducing the average experimental time consumption associated with each IC_{50} determination, and by employing alternative calculational algorithms. We have investigated the effect of the following three procedures on effective QPatch throughput:

1. Application of multiple drugs per cell
2. Reduction of the duration of the experimental protocol
3. Estimation of drug potency (IC_{50}) based on a single inhibitor concentration

MATERIALS AND METHODS

Cells: Cultured CHO cells expressing hERG potassium channels were used.

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 $MgCl_2$, KOH/EGTA 31.3/10, 10 HEPES, 120 KCl, 4 Na_2-ATP, pH=7.2 (KOH), ~290 mOsm.

Compounds: Bepridil (B5016), thioridazine (T9025), flecainide (I6777), tamoxifen (T5648), terfenadine (T9652), verapamil (V4629), pimozide (P1793), haloperidol (H1512) and quinidine (Q5004) were all from Sigma (Buchs, Switzerland). For single-concentration IC_{50} determinations were used concentrations of 0.1 and 1.0 µM.

Verapamil was used for voltage protocol optimizations in six concentrations from 0.003 – 300 µM in ten-fold increments.

Electrophysiology: Whole-cell patch-clamp experiments were performed on QPatch HT. Standard and experimental hERG voltage protocols are presented in Figure 2.

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). IC_{50} values based on single-concentration analysis were calculated according to Cheng & Prusoff (1973). Data analysis was accomplished with the QPatch Assay Software.

RESULTS AND DISCUSSION

1. Application of multiple drugs to each cell

An obvious way of increasing the throughput is to apply several compounds sequentially to each cell. We conducted a series of hERG experiments in which up to 16 compounds were added to each cell. Figure 1 shows seal and whole-cell resistances for the 48 channels of a single QPlate 48 used for a screening job. In this experiment 286 compound tests were initiated. Out of these 251 were completed successfully. The screening was completed in 43 minutes, and each cell was exposed to on average 5.6 compounds. As a mean 5.8 compounds were tested per minute. Assuming one job execution per hour the overall throughput comes to ~2000 compounds per day when defining 'one working day' as 8 hours, or, alternatively, 6000 compounds per 24 hours.
If the study had been conducted with only one compound per cell, six QPlate 48 would have been needed and the estimated time consumption is 2 hours.
In conclusion, by allowing multiple compound additions to each cell the throughput was approximately tripled.

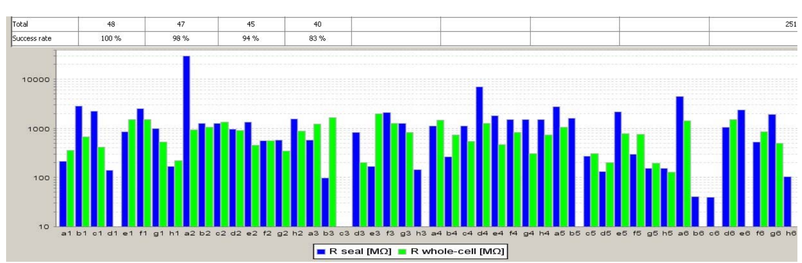


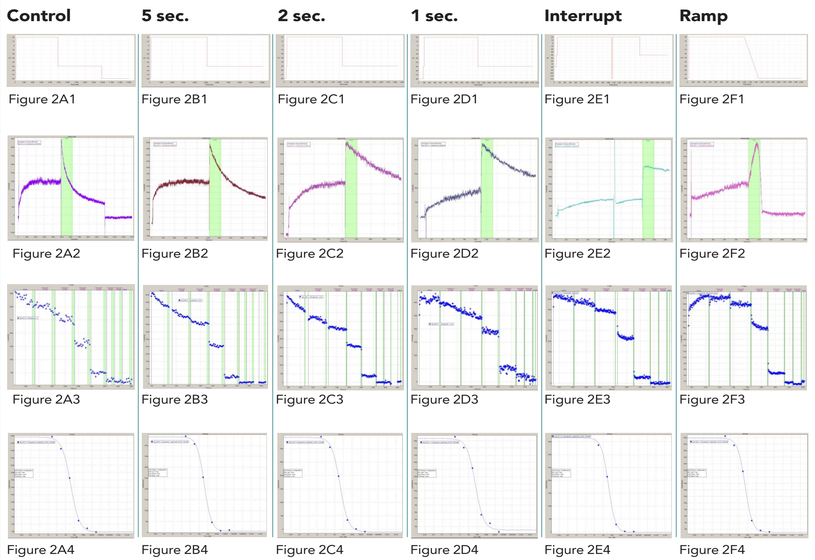
Figure 1

2. Reduction of the protocol execution time

In the standard hERG screening protocol (Figure 2A, Panel 1) the cell potential (V_c) is held at a holding potential (V_h) -80 mV for 80 ms. An initial brief (20 ms) depolarization to -50 mV (for leak current determination) is followed by a depolarization to a test potential (V_t) of +20 mV for 4800 ms. Subsequently, V_t is clamped to -50 mV for 5000 ms for tail current determination before being returned to -80 mV for 3100 ms. The total duration of the complete voltage protocol amounts to 13000 ms. Between each voltage protocol execution is inserted a 2000 ms pause. Consequently, the standard voltage protocol can be executed four times per minute.
The whole-cell current elicited in response to the voltage protocol is shown in the Panel 2.
Panel 3 shows the resulting current-time (I-t) plot throughout the control and the six compound periods.
Panel 4 shows the resulting Hill fit based on the steady-state currents presented in the I-t plot (Panel 3).

To increase the frequency of voltage protocol executions we tested the effect of shortening the duration of V_t. Subsequently we analyzed whether this procedure had any significant effect on the IC_{50} determinations. For this purpose three protocols were tested (Figure 2B-2D) with V_t of 5000, 2000 and 1000 ms, respectively. The total execution times for these protocols were 12100, 6100 and 4100 ms, respectively, corresponding to 5.0, 9.8 and 14.6 protocol executions per minute.

In addition we tested the two alternative short lasting protocols of 4100 ms duration that have been described in the literature. First, we tested an 'interrupt' protocol (Figure 2E) in which a 20 ms hyperpolarization to -120 mV were conducted during the depolarizing step (at 1060 ms) to release hERG channels from inactivation (Roden et al., 2002). This protocol proved advantageous because it led to an increased hERG current. Next, we tested a 'ramp' protocol in which V was continuously hyperpolarized from +20 mV to -80 mV in 250 ms (Brown, 2005), see Figure 2F.



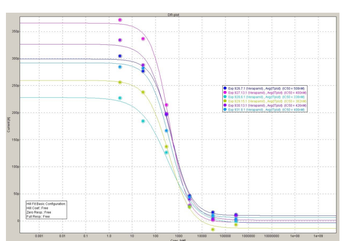


Figure 3A

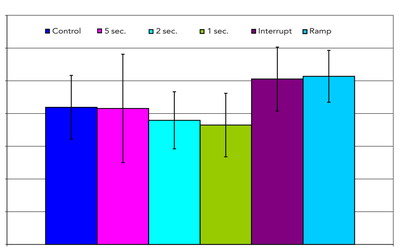


Figure 3B

For comparison of current amplitudes Figure 3A depicts Hill plots for each of the six voltage protocols collected in the same panel.

Importantly, without exception the IC_{50} values determined from the test protocols (Figure 2B-2F) were similar to the value obtained with the standard hERG protocol ('Control', Figure 2A1). A comparison between the IC_{50} values obtained with the six protocols is shown in Figure 3B.

In conclusion, without impairing data quality, a reduction of the protocol execution duration by a factor of three led to a tripling of the basic QPatch throughput for the compounds tested in this study.

3. Single-concentration IC_{50} determinations

IC_{50} values may be determined by an alternative procedure which requires only a single inhibitor concentration (Cheng and Prusoff, 1973). However, this concentration (C) needs to be within the interval $IC_{10}<C<IC_{90}$ for the method to be applicable. The method requires knowledge of the time constant for binding of the inhibitor to the substrate (hERG channel). This is obtained from an exponential fit of the I-t curve (Figure 4A). Thus, if binding is too fast (~instantaneous, beyond resolution) the method is ineffective. Figure 4B shows a plot of IC_{50} values obtained with the single-concentration method compared with values obtained with conventional determinations based on Hill plots. Numbers in parentheses denote number of experiments.

Black line is the line of identity (full agreement between the two sets of IC_{50} values). Red lines indicate a factor of 10 between the IC_{50} values. C was either 100 nM or 1 µM. It appears that for IC_{50} in the µM range the correlation is good whereas the more potent inhibitors (with IC_{50} in the nM range) yields poor results, illustrating that when C is outside the interval $IC_{10}<C<IC_{90}$ the method is not applicable. By reducing C to the nM range the correlation becomes good (not shown).
In conclusion, the single-concentration method can increase the throughput provided the concentration employed is comparable to IC_{50}.

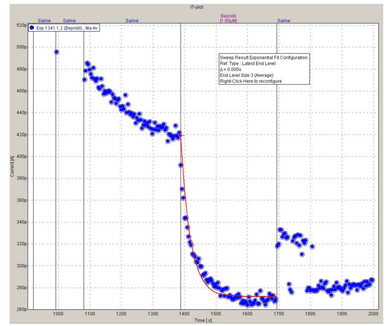


Figure 4A

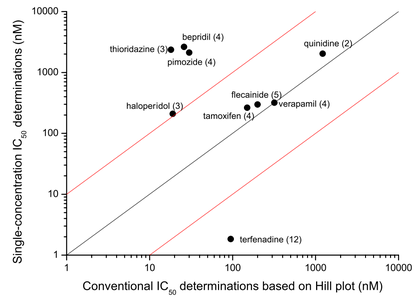


Figure 4B

SUMMARY

A substantial (approximately 10-fold) increase in QPatch throughput was achieved in a series of hERG studies by implementing three strategies:

1. multiple drug applications to each cell
2. shortening of the voltage protocol execution time by a factor of three without compromising the IC_{50} values.
3. single-concentration IC_{50} determinations

Strategies 1 and 2 were implemented without any precautions, whereas strategy 3 places strict requirements on the receptor-inhibitor interaction kinetics and on the magnitude of the test concentration. It is predicted that single-concentration IC_{50} determinations may become increasingly useful in fast lead optimization tests, in which compounds generally have fairly similar characteristics.

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