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Characterization of a hERG Screen Using the IonWorks HT: Comparison to a hERG Rubidium Efflux Screen

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Abstract: The introduction of parallel patch clamp instruments offers the promise of moderate-throughput, high-fidelity voltage clamp for drug screening assays. One such device, the IonWorks™ HT (Molecular Devices, Sunnyvale, CA), was evaluated and compared to conventional human *ether-a-go-go*-related gene (hERG) patch clamp data and an alternative functional screen based on rubidium flux. Data generated by the IonWorks HT and rubidium assays were compared to determine if either offered superior predictive value compared to conventional patch clamp. Concentration–effect curves for a panel of known hERG blockers were shifted to higher concentrations on the IonWorks HT compared to conventional voltage clamp determinations. The magnitude of the potency shifts was compound-specific and ranged from no shift (*e.g.*, quinidine) to over 200-fold (astemizole). When the extreme value for astemizole was disregarded, the potency shift for 13 other known reference standards was 12-fold or less, with an average shift of fivefold. The same subset of compounds in the rubidium efflux assay exhibited an average potency shift of 12-fold. To provide a simulation of how the IonWorks HT assay might perform in a single concentration screening mode, a panel of test compounds was evaluated. The IonWorks HT screen did not outperform the rubidium efflux screen in predicting conventional voltage clamp measurements. The most likely explanation appears to rest with variable and compound-specific potency shifts in the IonWorks HT assay. The variable potency shifts make it difficult to select a screening concentration that meets the criterion of a high positive predictive value while avoiding false-positives.

Introduction

THE POTASSIUM CHANNEL encoded by hERG (also known as KCNH2 or Kv11.1) is the pore-forming subunit for one of the major repolarizing potassium currents in human heart, I_{Kr}.[1] The unique voltage dependence, gating, and kinetics of I_{Kr} are ideally suited for a critical role in rapid repolarization of the cardiac action potential. On depolarization the channels open but rapidly inactivate, resulting in little outward current during the plateau phase of the cardiac action potential. During repolarization the channels recover from inactivation and then slowly close (deactivate).[2,3] This leads to a surge of outward current that contributes to the rapid repolarization phase of the cardiac action potential (reviewed by Tseng[4] and Vandenberg *et al*.[5]).

The functional significance of I_{Kr} for cardiac repolarization is clearly demonstrated in individuals with congenital loss of function of the hERG gene product. Mutations in hERG are responsible for one form of congenital long QT syndrome, which is associated with delayed cardiac repolarization, and an increased risk of *torsade de pointes*, ventricular fibrillation, and sudden death.[6,7]

As in individuals with congenital long QT syndrome, pharmacological block of I_{Kr} is also associated with prolonged QT intervals and an increased risk of arrhythmias and death.[8] Unintended block of I_{Kr} is a common side ef-

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ABBREVIATIONS: CHO, Chinese hamster ovary; DMSO, dimethyl sulfoxide; HEK, human embryonic kidney; hERG, human *ether-a-go-go*-related gene.

fect of drug candidates from many different chemical and pharmacological classes. Several unique structural features of the hERG channel are believed to contribute to the well-documented promiscuity of this channel. The inner vestibule of the channel is predicted to be wider for hERG than other voltage-gated potassium channels. There are also two hydrophobic residues (Y652 and F656) uniquely positioned in the inner cavity of the hERG channel that promote high-affinity binding of many known blockers.[9,10] MK-499, clofilium, and ibutilide have also been shown to interact with pore helix residues (T623, S624, and V625)[9,11]. Examples of marketed drugs that have been subsequently recognized as having I_{Kr} block as a side effect include the antihistamines terfenadine and astemizole[12]; the gastrointestinal prokinetic agent cisapride[13]; the antipsychotic agent sertindole[14]; and the antibiotics sparfloxacin and grepafloxacin.[15] Each of these drugs was developed before the scope and consequences of I_{Kr} block by xenobiotics were fully appreciated. After their pro-arrhythmic potential was better understood, all of these drugs were either removed from the market, or severe restrictions were placed on their use as therapeutics.

In order to avoid side effects related to block of I_{Kr}, early evaluation of I_{Kr} inhibition has become an integral part of most drug development efforts. Because of technical limitations of measuring native I_{Kr} currents and limited availability of human cardiac tissue, testing for block of human I_{Kr} current is usually conducted using model systems in which hERG is exogenously expressed in a stable manner in non-cardiac mammalian cell lines. Examples of commonly used background cell lines include HEK 293, CHO-K1, and L-929.[16–18] The most accurate and reliable procedure for measuring inhibition of hERG currents is voltage clamp using the whole-cell configuration of the patch clamp technique, but this suffers from the limitation of low throughput due to the labor-intensive nature of the work.

There are several established but indirect methods for evaluation of hERG liability that are amenable to higher-throughput techniques. These include competitive binding experiments with radiolabeled dofetilide or structurally related compounds,[19] voltage-sensitive fluorescent dye-based methods,[20] and rubidium efflux studies.[21] Although useful for screening, none of these indirect methods exhibits ideal potency or rank-order potency correlations with voltage clamp. An issue common to voltage dye and rubidium efflux methods is a compound-dependent decrease in measured potency using these assays compared to patch clamp measurements.[20] Displacement of radiolabeled dofetilide is a non-functional evaluation that is dependent on compounds of unknown activity binding to a site that overlaps the dofetilide binding site. For each of these screening assays, there is no direct control of the transmembrane potential, and hence no ability to cycle hERG channels through the voltage-dependent transitions between closed, open, and inactivated states that normally occur with each cardiac action potential. This is a major limitation because it has an impact on the ability of the screening assay to accurately capture the voltage- or state-dependent block that could occur in native cardiac cells.

There have been significant advances in recent years in the development of automated parallel patch clamp technologies. These devices offer the combined potential of uncompromised data quality comparable to that obtained using conventional whole-cell patch clamp, together with a greatly enhanced throughput. There are three parallel patch clamp devices that are presently marketed to end-users: the PatchXpress™ 700A (Molecular Devices, Sunnyvale, CA), the IonWorks™ HT (Molecular Devices, Sunnyvale, CA), and the Flyscreen® 8500 (Flyion GmbH, Tübingen, Germany). Several more automated patch clamp devices are in the final stages of development. Of the presently marketed parallel patch clamp devises, the IonWorks HT has the potential for the highest overall throughput because as many as 384 cells are evaluated in parallel, compared to either three or 16 cells for the Flyscreen 8500 and the PatchXpress 700A, respectively. A preliminary report suggests that the IonWorks HT is capable of faithfully capturing the potency of known hERG blockers, but the instrument's performance was not evaluated in a screening mode where compounds of unknown activity are typically evaluated at a single concentration.[22]

In the present study, we describe and compare our experience with a hERG screen that was developed using the IonWorks HT to data generated using a rubidium efflux assay and conventional whole-cell voltage clamp using 48 compounds from our library collection.

Materials and Methods

The IonWorks HT is a screening device for conducting parallel voltage clamp measurements. The disposable recording plates for this instrument (Patch Plate®) have 384 wells in an 8×48 layout (Fig. 1). Despite the 384-well consumable format, fluid addition occurs in only 12 wells at a time, and electrical measurements are made in only 48 wells simultaneously. The electronics head is arranged in a 2×24 electrode array with a two-well spacing between each of the 24 electrodes within a column and a four-well spacing between the two columns of electrodes. The fluidics head is a single column with 12 pipette tips. The spacing of one pipette tip to every fourth well on the patch plate is compatible with 96–well compound plates.

Each Patch Plate well has a 1–2 μm hole in the bottom on which cells can settle. Suction is continuously ap-

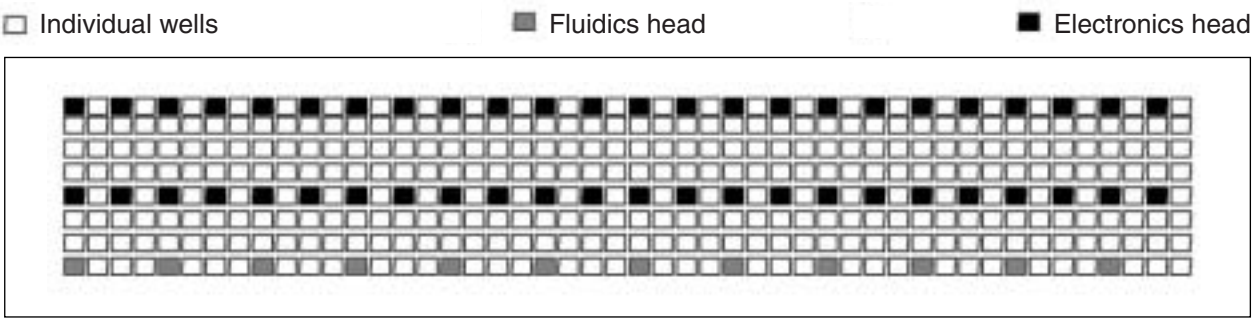

FIG. 1. Schematic representation of patch plate consumable and the spacing of the electronics and fluidics heads. Wells are arranged in an 8 × 48 array. Electrodes cover one-eighth of the plate at a time in a 2 × 24 array. Spacing of the electrodes is represented by the black wells. Fluid additions are made to 12 wells at a time. Spacing of the fluidics head is represented by the gray wells and corresponds to one row of a 96-well compound plate.

plied across the patch plate to promote the formation and maintenance of "seals." The manufacturer's definition of a "seal" is a resistance greater than 20 MΩ. User-defined filters can be used to enforce more stringent requirements for seal resistance. In the present study, electrical seal resistances ranged from a user-defined lower limit of 65 MΩ to approximately 250 MΩ. Data were also rejected if pre-compound hERG tail current amplitude was less than 150 pA or if the total resistance resulting from the parallel seal and membrane resistance decreased by more than 50% from the pre-compound measurement to the post-compound measurement.

Electrical access to the interior of all successfully positioned cells on the patch plate is achieved by the addition of the pore-forming antibiotic, amphotericin B, to a common chamber beneath the patch plate. For evaluation of the effects of test substances, cells are transiently voltage-clamped in blocks of 48 wells prior to addition of test substances and again after a user-defined incubation period. Test substances were added from a 96-well polypropylene compound plate. The contents of any one well of the 96-well compound plate are dispensed to four wells of the 384-well patch plate using on-board microfluidics. Overall success rates claimed for this instrument vary from 40% to 70% and depend on the channel of interest, the cell type, and assay conditions. The average success rate for the present 5-min, studies (wells passing all user-defined acceptance criteria; see above) was approximately 60%, with a range from 47% to 70%.

Cells

HEK 293 cells permanently expressing hERG in a stable manner (generated at Schering-Plough Research Institute) were selected for the final version of the IonWorks HT hERG screen. A hERG-CHO cell line (generated at Schering-Plough Research Institute) and a hERG-L929 cell line (subcloned from cells obtained from S. Taffit, State University of New York at Syracuse) were also evaluated on the IonWorks HT. hERG-L929 cells gave consistent results, but the optimum Z' factor (a measure of assay signal to noise)[22] and current amplitude obtained with this line were inferior to those observed with the hERG-HEK 293 cells. The following equation was used to calculate Z':

$$Z' = 1 - [(3SD_V + 3SD_D)/||V - D||]$$

where V = mean of the fraction of pre-drug current remaining in wells exposed to vehicle, D = mean of the fraction of pre-drug current in wells exposed to 10 μM dofetilide, and SD_V and SD_D = corresponding standard deviation for wells used to calculate V and D. In all cases, adherent cells were released from culture flasks using trypsin-EDTA (HEK 293 cells or CHO cells) or trypsin (L-929 cells). Cells were then pelleted and resuspended into Dulbecco's phosphate-buffered saline supplemented with 1.25 mM KCl.

Extensive preliminary experiments revealed appreciable differences in performance across each of the assay platforms between the three cell lines described above. We therefore selected different cell lines for each assay, with the criteria being optimum signal stability and success rate. The hERG-L929 cell line was used for conventional whole-cell patch clamp recording of hERG current. Optimum performance of the hERG Rb efflux screen was obtained with hERG-CHO cells. A hERG-HEK 293 cell line worked best for IonWorks HT studies. There were no obvious differences in hERG tail current kinetics among the three different host cell lines (Fig. 2). In addition, tail currents were blocked by approximately 95% by 100 nM dofetilide in all three cell lines (data not shown).

Voltage protocols

A limitation of the IonWorks HT is that cells are not continuously voltage-clamped. There is a transient period

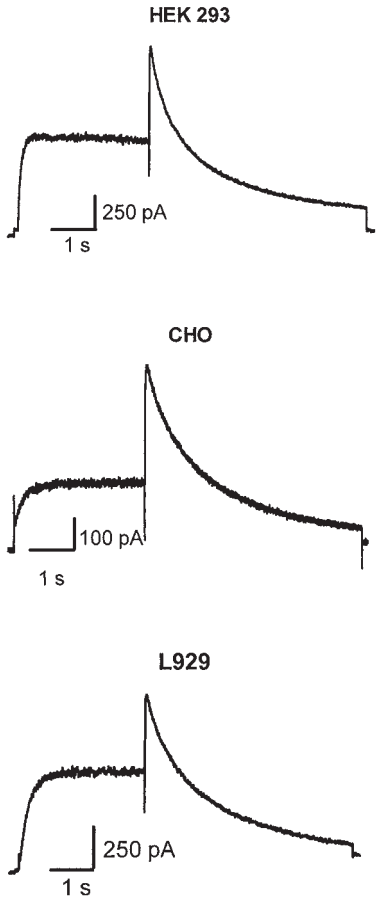

FIG. 2. Representative kinetics of membrane currents recorded from three different mammalian cell lines with stable expression of human *ether-a-go-go*-related gene (hERG). Conventional whole-cell recording was used to acquire these traces. In all three cases the hERG currents were activated with a 3-s depolarization to +20 mV, and tail currents were recorded during a 5-s repolarizing pulse to −60 mV. Holding potential was −70 mV. The step to +20 mV was preceded by a 200-ms step to −60 mV for the L929 and the human embryonic kidney (HEK) 293 cell but not for the Chinese hamster ovary (CHO) cell.

of clamp and data collection prior to compound addition and a comparable period of data collection at a user-defined interval after compound addition. For the IonWorks HT hERG studies, cells were clamped at −80 mV for 10 s prior to data collection to ensure maximal hERG channel availability. The current during a brief (200 ms) step to −40 mV was then sampled to provide a measure of all non-hERG currents (leak and other membrane currents). The measurement of this reference current was considered critical because we found the built-in leak subtraction algorithm used by the IonWorks HT software to be unreliable, possibly as a result of the low seal resistances obtained on the instrument. The 200-ms step to −40 mV was followed by a 5-s depolarization to +20 mV to activate the channels. Tail currents were measured during an ensuing return to −40 mV, and in all cases amplitude data refer to the peak tail current during the second step to −40 mV minus the non-hERG current at −40 mV. Representative hERG current traces from the IonWorks HT and the voltage protocol are shown in Fig. 3. The relatively lengthy (5 s) step to +20 mV was found to be critical to reduce potency shifts for some active-state blockers such as cisapride and dofetilide. For these two compounds IC_{50} values were two to three times higher when data from a 2-s activating pulse were compared to data from a 5-s activating pulse.

For whole-cell patch clamp studies hERG-L929 cells were plated onto a 9-mm circular coverglass and used within 5 days of plating; experiments were conducted at room temperature using a flow rate of 4 ml/min. The voltage clamp protocol used for conventional whole-cell patch clamp studies was similar to that used on the IonWorks HT, but the actual voltages and the duration of the steps differed. Non-hERG currents and hERG tail currents were measured at −60 mV, the depolarizing pulse to +20 mV was 3 s long, and cells were continuously voltage-clamped. In conventional whole-cell patch clamp studies hERG tail currents were repetitively measured every 10 s. Each cell in conventional patch clamp studies was exposed to only a single test intervention; this usually took the form of a single concentration of drug with measurements proceeding until a steady-state effect was observed, and in all cases control experiments used time-matched vehicle exposures. Percent inhibition in conventional whole-cell patch clamp studies was calculated relative to the mean of a control group of cells using the following equation:

$$\% \text{ inhibition} = [1 - (F_D/F_V)] \times 100\%$$

where F_D = the fraction of baseline current remaining after drug exposure, and F_V = the fraction of baseline current remaining after vehicle exposure. The fraction of current remaining after vehicle ranged from 0.90 to 1.0 with a trend for lower values with longer exposure times.

Rubidium efflux

hERG-CHO cells were plated into 96-well flat-bottom dishes and returned to the incubator for 24 h. On the day of study, culture medium was removed and replaced by 150 μl of a HEPES-buffered saline solution containing 5.4 m*M* RbCl (rubidium loading buffer), and the cells were then returned to the tissue culture incubator for 3 h to permit rubidium–potassium exchange. Test compounds were prepared at a fourfold final concentration in rubidium loading buffer containing 10% DMSO. Individual compounds were dis-

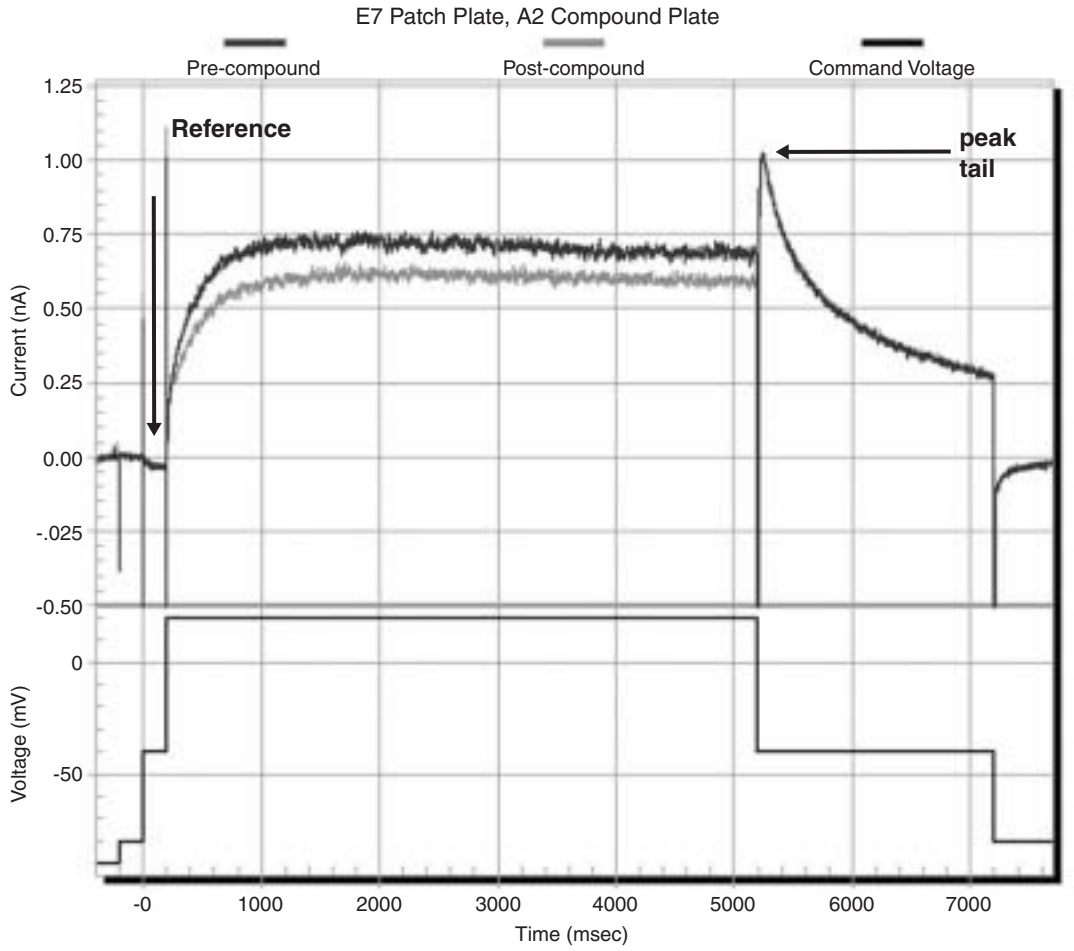

FIG. 3. Representative IonWorks HT current traces (**top**) in response to the voltage steps displayed at the **bottom**.

pensed (50 μl) into wells on the cell plate, returned to the incubator, and allowed to equilibrate for 30 min at 37°C. Cell plates were then washed three times with HEPES-buffered saline containing 5.4 m*M* KCl but no rubidium. After the final wash, cells were depolarized by the addition of 200 μl of HEPES-buffered saline containing 45.4 m*M* KCl. A 5-min depolarization was found to be adequate for efficient efflux of rubidium. Supernatants were collected and analyzed for rubidium content using automated flame atomic absorbance spectroscopy (ICR-8000 spectrometer, Aurora Biosciences, Vancouver, BC, Canada). Percent inhibition was quantified based on a signal window defined by vehicle (no block) and dofetilide (10 μM, full block) reference wells. Liquid handling for the rubidium efflux screen was implemented using a pipetting robot capable of making simultaneous additions and removals from 96 wells (Quadra 96, Tomtec, Hamden, CT).

Solutions

The external solution used for the IonWorks HT studies was Dulbecco's phosphate-buffered saline (Invitrogen, Carlsbad, CA) supplemented with 1.25 m*M* KCl to provide a final potassium concentration of 5.4 m*M*, 1 mg/ml glucose, and 1% DMSO. The internal solution contains 20 m*M* KCl, 130 m*M* potassium gluconate, 5 m*M* HEPES-KOH (pH 7.25), 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 1% DMSO. Amphotericin B was added at 5 mg in 65 ml when present (200 μl of DMSO was used to dissolve the amphotericin B prior to addition). The presence of 1% DMSO in all solutions did not affect current stability or well-to-well variability. Higher concentrations of DMSO were not evaluated. Compound plates were prepared as 3× because the IonWorks HT makes three 3.5-μl additions to each well (buffer alone, then buffer plus cells, then 3× compound). In screening mode, compounds were added from 1 mg/ml stocks in DMSO

by adding 2.5 μl of stock to 250 μl of DMSO-free saline per compound plate well. Screening plates were placed on a plate shaker for at least 20 min prior to being positioned on the IonWorks HT. Compound plates for concentration–effect curves were prepared by half-log serial dilution of compounds in saline containing 1% DMSO.

The buffer for patch clamp studies was 144 m*M* NaCl, 11 m*M* glucose, 10 m*M* HEPES-NaOH (pH 7.35), 5.4 m*M* KCl, 1.8 m*M* $CaCl_2$, and 1.0 m*M* $MgCl_2$. The pipette solution was 140 m*M* KCl, 10 m*M* HEPES-KOH (pH 7.2), 3 m*M* MgATP, 5 m*M* EGTA, and 0.3 m*M* $MgCl_2$.

The base solution for the rubidium efflux studies was 144 m*M* NaCl, 20 m*M* HEPES-NaOH (pH 7.4), 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 11 m*M* glucose. Drugs, KCl, and RbCl were added to this base solution as indicated above.

Results

Two of our three hERG cell lines (hERG-HEK 293 and hERG-L929) were found to be amenable to the IonWorks HT platform. However, the ability to perform a large number of simultaneous voltage clamp determinations using IonWorks HT quickly indicated that neither of these cell lines was homogeneous with regard to hERG expression. Subcloning both lines improved overall success rates with both cell lines because of an increase in the fraction of cells meeting the minimum current acceptance criteria. The IonWorks HT instrument greatly simplified the clonal selection work because 12 clones can be evaluated during a run, permitting a single investigator to handle the evaluation of at least 60 subclones per day. After comparing the best hERG-HEK 293 subclone to the best hERG-L929 subclone, for stability over time and signal-to-noise, an HEK 293 subclone was selected for further assay development.

Preliminary experiments also identified a compound carryover effect on the IonWorks HT that needed to be resolved before routine assays could be implemented. The carryover appeared to be due to compound adhesion to the permanent pipetting tips on the fluidics head of the IonWorks HT. The same tips are used for all fluid additions to the wells, including buffer to prime the wells, cell suspension and compounds. The carryover effect was clearly demonstrated in experiments where the compound plate contained a high concentration of a "sticky" hERG blocker in the first row and buffer in all subsequent wells. When such a compound plate format was generated using 10 μM astemizole in the first row, it was apparent that there was residual hERG block by nominal buffer additions for several wells after the addition of drug from the first row (Fig. 4, top). There is also an increase in the variability of the data. Similar results were observed with pimozide. The carryover issue was resolved by the manufacturer with installation of a software upgrade (IonWorks HT software version 1.3) that permitted the fluidics tips to be washed with DMSO followed by a buffer wash in between each addition of compound. With the DMSO wash, no carryover of astemizole or pimozide was observed (Fig. 4, bottom). It should be noted that although the DMSO wash step eliminates inadvertent carryover of compound, a potential loss of compound to the pipetting tips remains an issue.

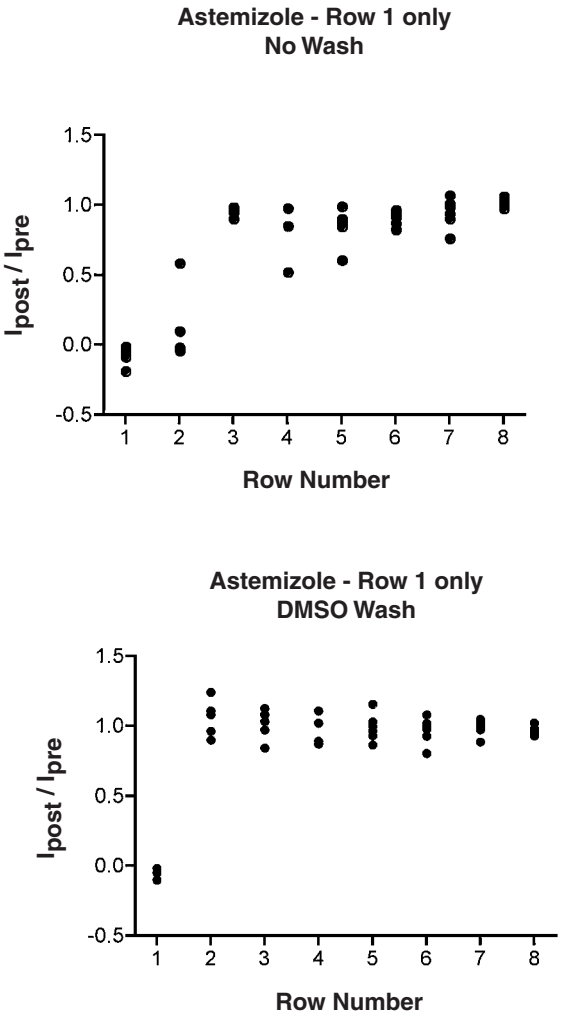

FIG. 4. Carryover of astemizole on the IonWorks HT. Astemizole was present in only the first row of the compound plate. **Top:** Without wash of the fluidics tips there is residual inhibition of human *ether-a-go-go*-related gene current after addition of drug-free buffer in rows 2, 4, and 5. Also note the increased variability. I_{post} = current after addition of test substance; I_{pre} = baseline current. **Bottom:** Fluidics tips washed with dimethyl sulfoxide (DMSO) followed by buffer in between additions from the compound plate. Data are from successive runs on the same day.

Screen performance

After optimization, the final IonWorks HT hERG protocol that was adopted exhibited reasonable stability for

5 min after vehicle addition and a good signal-to-noise; Z' factor determinations ranged from 0.40 to 0.63. Examples of data used for Z' and IC_{50} calculations are shown in Fig. 5. The performance of the IonWorks HT assay was assessed by comparing potency data generated for a number of reference compounds to data from conventional whole-cell patch clamp experiments. Patch clamp reference IC_{50} data are listed in Table 1. The potency of two of the reference compounds, quinidine and E-4031, was accurately reproduced on the IonWorks HT. For the remaining 12 reference compounds there was a right shift in potency. Astemizole was an extreme case, with a potency shift of 242-fold. The remaining compounds exhibited potency shifts of less than 12, with an average shift of fivefold (astemizole excluded) (Table 2). Although currents were found to be stable for 10 min after addition of vehicle, no potency advantage was obtained by prolonging compound exposure time from 5 min to 10 min. The same subset of compounds exhibited an average potency shift of 12–fold in the hERG rubidium efflux screen.

Based on the potency information, the IonWorks HT assay was evaluated in a simulated screening mode using a fixed concentration of proprietary compounds for which data were available from both conventional voltage clamp (1 μM for most but 5 μM for one chemical series) and hERG rubidium efflux at 5 μg/ml. The initial screening concentration on the IonWorks HT was 2 μg/ml, but this was subsequently increased to 3.3 μg/ml in order to reduce the false-negative rate. The IonWorks HT screen correctly predicted 30 of 48 compounds (62.5%) (Table 3) when an arbitrary criterion

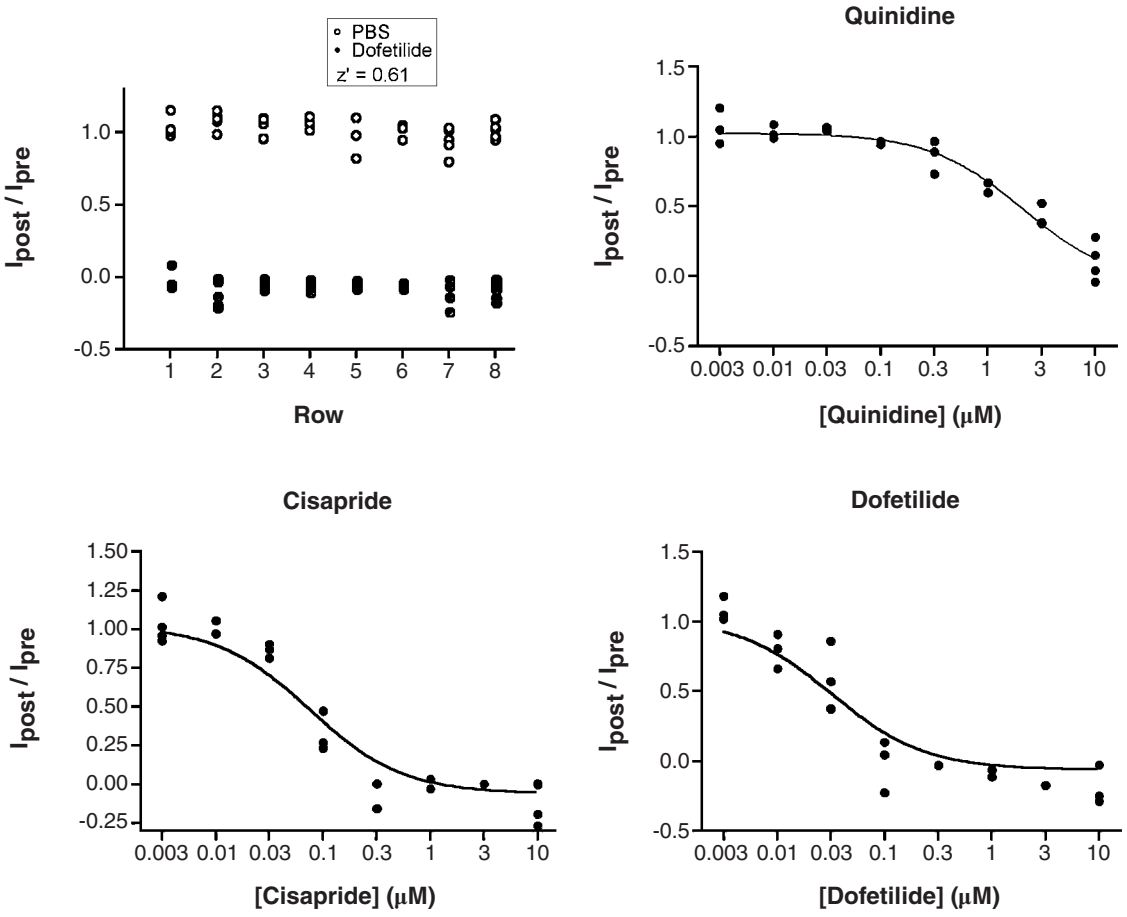

FIG. 5. IonWorks HT current stability and representative concentration–effect relationships. Compound plates were set up with one column containing buffer (phosphate-buffered saline [PBS]), one column containing 10 μM dofetilde, and the remaining columns containing half-log increments in the concentration of standard human *ether-a-go-go*-related gene blockers, with the lowest drug concentrations in row 1 and the highest concentration in row 8. Theoretical fits to the following equation were generated in GraphPad Prims version 3.0: $y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10\ [(\log EC_{50} - x)])$, where x is the logarithm of concentration and y is the response. The top and bottom values were calculated as the mean of the buffer wells and the mean of the dofetilide wells, respectively. These values were then constrained for all of the concentration–effect curves on the corresponding plate.

of being within 30% of the value determined by voltage clamp was applied. Of the 18 compounds that missed by >30%, three were false-positives. A similar comparison for the rubidium efflux assay using data generated from the same compound plates that were used for the IonWorks HT assay showed that Rb efflux correctly predicted 32 of 48 compounds (66.7%). None of the compounds showed ≥30% higher activity in the rubidium efflux assay compared to conventional whole-cell patch clamp. For 24 of the 48 compounds both assays came within 30% of the voltage clamp activity. The efflux assay missed six compounds that were within 30% using the IonWorks HT platform. The IonWorks HT assay missed eight compounds that were within 30% in the efflux assay.

An alternative means to compare the two higher-throughput assays to voltage clamp data is to look at the correlation between the data sets. This analysis revealed the correlation coefficient to be higher for the Rb efflux data versus voltage clamp ($r = 0.45$) than for the IonWorks HT data versus voltage clamp data ($r = 0.27$) (Fig. 6). It is also apparent from the graph that the lower correlation between the IonWorks HT and voltage clamp cannot be attributed solely to the three false-positives observed with the IonWorks HT.

Discussion

Several potential sources of compound potency shifts are likely to apply to each of the two high-throughput screening assays that were evaluated in the present study. Sources of error relative to conventional whole-cell patch clamp that are common to both assays include: (a) Microgram per milliliter concentrations were used in the screening assays, and a fixed micromolar concentration in conventional whole-cell patch clamp measurements. Due to differences in formula weight (range 250–750) the range of micromolar concentrations in the IonWorks HT screen is approximately 4.4–13.2 μM. (b) Polypropylene compound plates were used as drug reservoirs in the case of the screening assays. It must be considered likely that there will be some hydrophobic compounds that are depleted from solutions on the compound plate due to adsorption to the polypropylene matrix. Adsorption to pipetting tips is also likely to be a source of error. The carryover observed for the IonWorks HT when there is no DMSO wash step between compound wells is evidence that there must also be loss of some compounds to the pipetting tips on the fluidics head of the IonWorks HT. Finally, the plastic wells of the IonWorks patch plate have a high surface-to-volume ratio, and compound depletion by adsorption to the patch plate might also occur.

Compound exposure time is also problematic with the IonWorks HT because the variability of replicate wells can increase with longer exposure times, resulting in a reduced Z' factor. Although this exposure time constraint could result in an underestimate of the activity of compounds with a slow on-rate, we did not observe any change in the IC_{50} values for known hERG blockers when the exposure time was increased from 5 to 10 min.

The potency of compounds exhibiting use-dependent block could also have been underestimated in the IonWorks HT protocol that we used because there was only

Table 1. Conventional Patch Clamp IC_{50} Values

Compound	*Voltage clamp IC_{50} (nM)*	*Reference*
Dofetilide	6	[a]
Terfenadine	56	Rampe *et al.*[13]
Amiodarone	1,000	Yang *et al.*[23]
Compound A	43	[a]
Pimozide	18	Kang *et al.*[24]
Bepridil	550	Chouabe *et al.*[25]
Nefazadone	270	[a]
Quinidine	1,000	[a]
Astemizole	1	Zhou *et al.*[16]
E-4031	31	[a]
Compound B	1,300	[a]
Compound C	92	[a]
Cisapride	9	[a]
Verapamil	830	Chouabe *et al.*[25]

[a]Schering-Plough Research Institute data.

Table 2. Potency Shifts Compared to Conventional Patch Clamp

	Rb IC_{50}/ VC IC_{50}	*IW IC_{50}/ VC IC_{50}*
Dofetilide	8.1	3.4
Terfenadine	18.4	10.1
Amiodarone	2.1	7.1
Compound A	18.9	7.0
Pimozide	2.2	11.6
Bepridil	1.8	1.6
Nefazadone	18.5	7.4
Quinidine	17.1	1.0
Astemizole	136.7	242.5
E-4031	16.6	0.9
Compound B	11.5	4.6
Compound C	7.3	1.8
Cisapride	28.8	8.3
Verapamil	8.7	2.0
Average	21.2	22.1
Average without astemizole	12.3	5.1

VC, conventional voltage clamp; IW, IonWorks; Rb, rubidium efflux.

Table 3. Performance in Screening Mode

SCH number	*Average % inhibition IW*	*% inhibition Rb efflux*	*VC 1 μM*	*% inhibition VC 5 μM*	*Comments*
Compound 1	31	72	82		a
Compound 2	11	54	73		a
Compound 3	69	44	19		a
Compound 4	−6	25	54		a
Compound 5	54	40	16		a
Compound 6	58	51	23		a
Compound 7	32	39	66		a
Compound 8	4	11	40		a
Compound 9	89	53		97	b
Compound 10	32	0		51	b
Compound 11	25	8	43		b
Compound 12	74	48		92	b
Compound 13	74	25	61		b
Compound 14	71	32	74		b
Compound 15	−2	29	72		c
Compound 16	2	−12	44		c
Compound 17	1	52	98		c
Compound 18	2	4	89		c
Compound 19	38	23	85		c
Compound 20	30	4	72		c
Compound 21	27	3		60	c
Compound 22	10	14	73		c
Compound 23	30	34	69		c
Compound 24	9	15	57		c
Compound 25	−4	15	30		
Compound 26	7	5		33	
Compound 27	11	−11	13		
Compound 28	96	101	99		
Compound 29	25	−3	10		
Compound 30	71	70	86		
Compound 31	9	6	15		
Compound 32	63	60	85		
Compound 33	91	74		100	
Compound 34	69	70	85		
Compound 35	13	6	26		
Compound 36	89	85	98		
Compound 37	0	−1	12		
Compound 38	6	5	33		
Compound 39	−2	2	13		
Compound 40	16	8	16		
Compound 41	17	44	26		
Compound 42	−5	16	23		
Compound 43	18	4	9		
Compound 44	23	20	40		
Compound 45	25	4	13		
Compound 46	86	44	71		
Compound 47	12	0	27		
Compound 48	16	6	19		

IW, IonWorks HT; VC, voltage clamp. For comments: a = IW miss >30%, Rb OK; b = Rb miss >30%, IW OK; c = both miss VC by >30%. Negative activity values assumed = 0.

a single cycle of channel activation after exposure to test interventions. When a protocol using three depolarizing steps was compared to a protocol with a single depolarization step, none of the standard compounds (see Table 2) exhibited more block on the third pulse compared to the first pulse. However, this does not completely exclude the possibility that we might have underestimated the block by some of the screened compounds should they preferentially block open or inactive states of the hERG channel with a slow on-rate.

State-dependent block could also limit the potency estimates in the rubidium efflux screen. Channels are

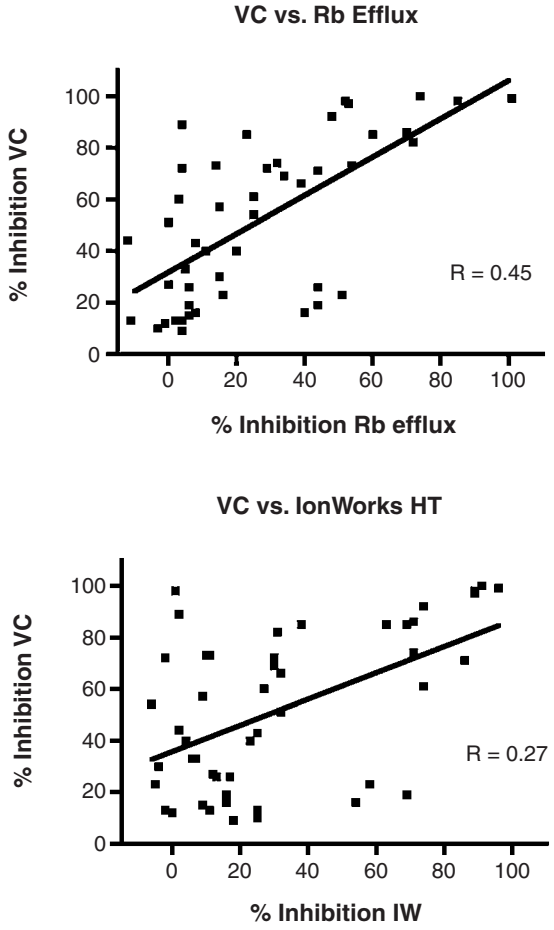

FIG. 6. Correlation with conventional whole-cell patch clamp data. IW, IonWorks HT; VC, voltage clamp.

opened by depolarizing cells with 45.4 m*M* extracellular potassium. Assuming the cell membrane potential changes in accordance with the potassium equilibrium potential, one would predict a transmembrane potential of −29 mV. At this potential, open channels are beginning to accumulate, but there would be a lower percentage of open and inactivated channels in the rubidium efflux screen than in either the IonWorks HT screen or conventional patch clamp studies. Another confounding factor in the rubidium efflux screen is that the potency of some drugs is known to be reduced by elevation of extracellular potassium.[26] A recently identified limitation of hERG rubidium efflux screens is a reduction in the potency of many compounds when rubidium replaces potassium as the permeant ion.[27]

The composite effect of all the assay limitations discussed above will have an impact on the predictive value of the screen. On balance, despite the advantages of achieving a degree of control over transmembrane potential and the use of physiological potassium concentrations, the IonWorks HT did not outperform a rubidium efflux-based assay for hERG liability. For both assays, variable and compound-dependent potency shifts complicate selection of a screening concentration. The main sources of error on the IonWorks appear to be related to loss of compound and limited recording stability. The manufacturer is continuing to develop the IonWorks HT, and it is possible that future improvements to the instrument will reduce the impact of the limitations we have discussed above. Changes enabling a reduction in adsorptive loss of compound to the fluidics head and the patch plate would be particularly beneficial.

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