IonWorks™ HT: A New High-Throughput Electrophysiology Measurement Platform

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To address the throughput restrictions of classical patch clamp electrophysiology, Essen Instruments has developed a plate-based electrophysiology measurement platform. The instrument is an integrated platform that consists of computer-controlled fluid handling, recording electronics, and processing tools capable of voltage clamp whole-cell recordings from thousands of individual cells per day. To establish a recording, the system uses a planar, multiwell substrate (a PatchPlate™). The system effectively positions 1 cell into a hole separating 2 fluid compartments in each well of the substrate. Voltage control and current recordings from the cell membrane are made subsequent to gaining access to the cell interior by applying a permeabilizing agent to the intracellular side. Based on the multiwell design of the PatchPlate™, voltage clamp recordings of up to 384 individual cells can be made in minutes and are comparable to measurements made using traditional electrophysiology techniques. An integrated pipetting system allows for up to 2 additions of modulation agents. Typical throughput, measurement fidelity, stability, and comparative pharmacology of a recombinant voltage-dependent sodium channel (hNav1.3) and a voltage-gated potassium channel (hKv1.5) exogenously expressed in CHO cells are presented. The IonWorks™ HT device can be used in biophysical and pharmacological profiling of ion channels in an environment compatible with high-capacity screening. (Journal of Biomolecular Screening 2003;50-64)

Key words: Potassium channels, sodium channels, electrophysiology, drug screening

INTRODUCTION

Advances in technology have been an important contributor to increasing the understanding of many biological systems. For ion channels, improvements in electrophysiological methods have created the opportunity to study the functional activity of a wide variety of ion channels in native tissues, as well as reconstituted cell and cell-free systems.1 The first recordings of passive membrane properties and propagated action potentials in squid giant axon2-7 defined many of the basic principles by which more advanced ion channel functional measurements have been built. In particular, the voltage clamp technique was developed to separate the capacitative and resistive properties of the cell membrane to permit measurement of membrane currents that result from the opening and closing of ion channels. Initial techniques to control membrane voltage and record ion channel currents involved the use of large-diameter wires inserted along the length of squid giant axons. These early techniques have evolved into more conventional practices, including the use of 2 microelectrodes to voltage clamp Xenopus oocytes and tissues as well as the use of single microelectrodes in the patch clamp technique.8 In recent years, a considerable amount of ion channel research has focused on identifying and characterizing ion channel genes and isolated cells, which has led to the widespread use of the patch clamp and 2 microelectrode voltage clamp techniques. Implementation of these methodologies has led to an explosion of knowledge of ion channel structure, function, and pharmacology.

The method of whole-cell recording described by Hamill et al.8 has been used to record ion channel activity from small cells.9 In this configuration of the patch clamp technique, a high-resistance seal forms at the interface of the cell membrane and small-diameter glass pipette. Subsequent to seal formation, the membrane across the orifice of the pipette is broken, thereby allowing access to the interior of the cell. This procedure allowed for measurement of electrical signals from the membrane of small cells and was distinguished from the previous recording methods for larger cells that employed dialysis10,11 by its high-resistance seal. This tight-seal,
whole-cell recording has been instrumental in measuring ion channels in a variety of excitable and nonexcitable cells.

Although conventional electrophysiological techniques permit the most direct and detailed study of ion channels, the disadvantage of these methods is the high level of manual intervention required and the corresponding low measurement throughput. Dependent on a variety of factors, including the cell and the type of ion channel under examination, an experienced electrophysiologist can make measurements on < 10 individual cells per day. Despite previous attempts to increase capacity, the patch clamp technique has thus far not been amenable to higher throughput. This has proven to be a significant limitation in the quest to discover new ion channel genes, novel physiology, and new ion channel drugs.

The goal of the high-throughput electrophysiology instrumentation described in this report was to create a technology that couples the high-fidelity measurements made by a standard patch clamp device with the measurement throughput required to support high-throughput screening, thus increasing the number of cellular measurements by > 100-fold. Achieving this goal required more than the automation of existing patch clamp techniques; it required the development of an entirely new paradigm for making electrophysiological measurements. The technology used allows a single cell to reside on a hole at the bottom of a well separating 2 aqueous compartments. The reservoir defined as the exterior of the cell is accessible to an electronics head capable of recording from 48 wells simultaneously and to a fluids head capable of dispensing < 1 µL of solution. The reservoir defined as the interior of the cell contains an electronic ground and is used to perforate the cell to allow recording of the ion channels in the cell membrane. Using this device, the biophysical and pharmacological characteristics of voltage-dependent potassium and sodium channels were demonstrated.

MATERIALS AND METHODS

Cell maintenance and preparation

Stable cell lines expressing hKv1.5 or hNav1.3 channels were created by transfecting CHO cells with the appropriate vector (pcDNA3.1 for Kv1.5, pCIN5 for Nav1.3)\textsuperscript{12,13} containing a neomycin-selectable marker with the CMV promoter, using the lipofectamine (Invitrogen, Carlsbad, CA) method. Cells were cultured in Kaign’s modified F-12 K media (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (ATCC, Manassas, VA), 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA), and 1% G418 (Invitrogen, Carlsbad, CA), and they were maintained at 37 °C in a humidified environment containing 6% CO\textsubscript{2} in air. Cells were liberated from the culture flask for passage and harvesting using Versene (Invitrogen). Immediately prior to use in the IonWorks\textsuperscript{TM} system, adherent cells were placed into suspension by first treating with Versene for 6 to 7 min at 37 °C, gently tapping the flask, resuspending in media or phosphate-buffered saline (PBS) (Gibco 14040-133, Invitrogen, Carlsbad, CA), and then centrifuging at 50 × gravity for 4 min. After a brief trituration, cells were finally resuspended at a concentration of approximately 1 × 10\textsuperscript{6} cells mL\textsuperscript{-1} in the external recording solution.

Conventional patch clamp data

Adherent cells were placed in a recording chamber mounted on the stage of an inverted microscope. All solutions and experiments were performed at room temperature (19-21 °C). Patch pipettes were pulled using thin-wall glass (TWF-150, World Precision Instruments, Sarasota, FL) and had resistances of approximately 2 MΩ when filled with a recording solution consisting of, in mM, the following: KCl, 140; HEPES, 1; MgCl\textsubscript{2}, 1; EGTA, 1; pH 7.2. They were then immersed in the phosphate-buffered saline salt solution (PBS 14040-133, Invitrogen, Carlsbad, CA). The whole-cell configuration of the patch clamp technique was employed by rupturing the membrane with suction and electroporation “zapping.” Whole-cell patch clamp recordings were made using an Axopatch 1D amplifier and a Digidata 1200A A/D converter connected to a personal computer. Data acquisition was performed under the control of pClamp (Axon Instruments, Union City, CA), and data analysis was made using a combination of pClamp and Excel (Microsoft, Redmond, WA).

IonWorks\textsuperscript{TM} HT technology

The design concept of IonWorks\textsuperscript{TM} HT is illustrated in Figure 1. The technology is based on positioning a cell on a small hole separating 2 isolated fluid chambers (Fig. 1A) in a manner that requires no manual intervention or micromanipulation. First, a high-resistance seal is formed between the cell membrane and peripheral region of the substrate pore. Second, a low-resistance electrical pathway is formed via the cell wall that covers the pore, thus permitting electrical access to the cell’s interior. These conditions permit the membrane potential over the remainder of the cell membrane to be voltage clamped and ionic currents from the cell plasma membrane to be measured. Given this basic measurement geometry, it is then possible to conceive of a parallel format in which many wells can be measured simultaneously (Fig. 1B). The IonWorks\textsuperscript{TM} HT system employs such a format in the form of the device illustrated in Figure 1C. The PatchPlate\textsuperscript{TM} is constructed as a single-use consumable and is a planar 384-well substrate arranged in an 8 × 48 well array. The spacing of the array is equivalent to a 1536 microplate (2.25 mm), and each well can hold up to 15 µL. Each well of the PatchPlate\textsuperscript{TM} contains a centrally located microhole and allows communication between the upper and lower portions, defined operationally as the extracellular and intracellular compartments, respectively. The well format was obtained by binding an injection-molded carrier structure to the thin planar substrate. The holes are microfabricated in the substrate sheet by a high-precision laser.
IonWorks™ HT device

Figure 2A is a picture of the IonWorks™ HT instrument. The basic components of the system include the experiment platform, the system pipettor, an intracellular fluidics system, an electronics recording head, the imbedded system control computer, and the user interface computer. Figure 2B is a close-up view of the experimental platform. The system pipettor is used to transfer extracellular buffer, cells, and experimental compounds from the respective source positions to the top-side wells of the PatchPlate™. The pipettor is a parallel 12-channel design and has < 5% coefficient of variation (CV) at 3.5 µL dispense volumes.

The intracellular fluidics system consists of several subsystems, including the PatchPlate™ clamp system, the PatchPlate™ reservoir or plenum, a vacuum regulation system, and a series of peristaltic pumps. The purpose of the intracellular fluidics system is multifold. First and foremost, this system provides a means to prime and debubble the bottom side of the PatchPlate™ with intracellular saline solution. The vacuum regulation system introduces a differential pressure across the substrate, thereby creating fluid flow through the microhole. This fluid flow positions cells introduced in the upper extracellular compartments of the PatchPlate™ onto the individual microholes in the substrate. As part of this system, the plenum reservoir houses a Ag/AgCl electrode that serves as a common ground to all the PatchPlate™ wells. Last, the intracellular fluidics system allows for the exchange of the intracellular solution with a second solution (e.g., a perforation agent solution) while maintaining a very precise differential pressure across the substrate.

The electronics recording head is composed of an integrated 48-channel transimpedence amplifier. Each channel is capable of controlling transmembrane voltage and measuring current via an Ag/AgCl-coated pin. Access to all 384 wells of the PatchPlate™

FIG. 1. IonWorks™ HT design concept. (A) depicts the basic measurement geometry, (B) extends this geometry to a parallel grid, and (C) is a picture of a 384 PatchPlate™.
occurs by moving the 48-channel head in 8 successive move/read steps. The maximum sampling frequency of the instrument is 10 kHz, and the RMS noise for each channel is < 10 pA.

The function of the instrument is under computer control via a graphical user interface (GUI) run on a system interface computer. High-level commands are relayed via an Ethernet interface to an embedded computer that controls time-critical aspects of system operations. Experimental protocols controlling the number and nature of current recordings, their timing in association with compound additions, and the timing and introduction of a perforation agent are all user definable. As an example, the command voltage protocol applied to the cells is defined via a graphical waveform editor dialog box, which, aside from the waveform itself, allows for the definition of sampling frequency, hold times, and repetition of a voltage waveform (see Fig. 3). The measured current from each of the 48 channels is displayed to the user in quasi-real time as a 48-channel display (see Fig. 4). Depending on the voltage protocol, the entire 384-well plate can be read in as fast as 30 s.

**IonWorks™ basic operation protocol**

To run an experiment, the user loads a PatchPlate™, the compound plate, and the cell suspension onto the experimental platform. The control software then takes the instrument through a series of steps designed to culminate in measuring currents from cells under voltage control. First, the system pipettor primes the PatchPlate™ by adding extracellular saline solution to the top side. The intracellular fluidics system then primes and debubbles the planar substrate, introducing intracellular solution to the bottom side. Once primed, the recording head steps across the plate, applying a small voltage waveform and in turn measures the current for each respective well. These measurements are used to quantify the electrical resistance for each well, determined primarily by microhole diameter. The pipettor then adds 3.5 µL of the cell suspension to each well of the PatchPlate™, and individual cells are guided to the respective microhole by differential pressure applied across the substrate. Once a cell reaches the microhole, a high-
resistance seal forms. Resistances of a few hundred MOhms are typical, form in < 5 min, and are stable for >1 h. Once the seal has formed, a perforating agent such as amphotericin or nystatin is added to permit electrical access to the cell interior. Access resistances between 5 and 10 MOhms are available in ~6 min and permit stable and accurate voltage control. At this stage, physiological recordings are made and experimental compounds added according to user-defined protocols.

Drugs

Final concentrations of all ion channel active compounds were obtained by dilution of the working stock (~10 mM in DMSO) into the indicated external solution. Regardless of the electrophysiology platform used to evaluate compound action, DMSO levels did not exceed 1%. Drug concentration-response curves were fitted to a 4-parameter equation of the form $y = (A_1 - A_2)/(1 + (x/x_0)^p) + A_2$, where $A_1 =$ maximum, $A_2 =$ minimum, $x_0 =$ IC$_{50}$ value, and $p =$ slope value.

RESULTS and DISCUSSION

Voltage clamp capability

Figure 5 examines the performance of the IonWorks™ HT system compared to a classical or conventional patch clamp using CHO cells stably transfected with the hKv1.5. Data shown correspond to the measured currents for the conventional patch clamp system (Fig. 5A) with respect to the IonWorks™ HT system (Fig. 5B). A family of step protocols is shown representing the command test voltage, which varied from a holding potential of ~70 mV to the indicated depolarized level and held for 90 msec before returning to ~70 mV. By measuring the current that is generated at each depolarized step potential, a current-voltage plot is generated for hKv1.5, using each technology (Fig. 5C). Note the similarity in current waveforms and current-voltage relationship. Thus, under the same experimental conditions, the IonWorks™ HT system has comparable performance to traditional patch clamp techniques.
A further illustration of response time and voltage clamp capabilities of IonWorks™ HT is illustrated in Figure 6, using the hNav1.3 channel stably expressed in CHO cells. The membrane currents measured using a classical patch clamp system (Fig. 6A) and the IonWorks™ HT system (Fig. 6B) are directly compared. For these experiments, test potentials were sequentially depolarized from +40 to –40 mV from a constant holding voltage (–90 mV) every 10 s. For each device, the measured currents demonstrate similar activation and inactivation current waveforms. The resulting current-voltage relationships (see Fig. 6C) are very similar. Note that, in both examples, no resistive or capacitative compensation was applied.

In addition to hKv1.5 and hNav1.3 channel currents, human Kv3.2, Kv2.1, and HERG expressed in CHO and HEK cells have been successfully recorded using the IonWorks™ HT system. In each case, the electrophysiological and pharmacological properties were well matched when compared to classical patch clamp techniques (data not shown). Taken together, the data show that the IonWorks™ HT system has essentially the same voltage clamp, time resolution, and signal-to-noise capability as a classical system.

**Success rates**

The measurement throughput achieved by the IonWorks™ HT platform depends on many factors. Ultimately, throughput is governed by a successive physiological measurement in response to the application of a potential pharmacological agent. Typically, this occurs by performing a physiological “preread” measurement on all of the wells of the PatchPlate™ followed by a test compound addition, which in turn is followed by an electrical “postread.” Using this format, each precompound measurement can be used as its own control, thereby adjusting for cell-to-cell variation in ion channel expression. Just as in a classical patch clamp system, and

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**FIG. 5.** Comparative biophysics of CHO-hKv1.5 K⁺ currents, as measured by conventional patch clamp electrophysiology and Ionworks™ HT. (A) Patch clamp and (B) Ionworks™ HT families of currents obtained using the voltage pulse protocol displayed at the foot of the figure. The horizontal and vertical calibration bars are 10 ms and 3 nA, respectively. (C) The peak current-voltage relationship for the 2 cells shown in (A) and (B).
given the variable nature of single-cell measurements, 100% success rates are not obtainable. Several factors contribute to failures, such as unsuccessful priming of the individual PatchPlate™ well, unacceptable seal resistance, inability to achieve electrical access, nonexpression in the given cell, and lack of the ability to establish a "stable" physiological recording before, during, and subsequent to the compound addition. Table 1 summarizes the typical statistical performance of the IonWorks™ HT platform for the kV1.5 and hNav1.3 cells. As shown, overall performance for stable physiological measurements is on the order of 60% to 80%, depending on the cell line and channel expression rates. Given these rates, a technician can perform more than 3500 successful patches per 8-hour day.

Redundancy

Because the success rate of achieving a stable physiological recording for each well is not 100%, a certain amount of compound sampling redundancy has been built into the IonWorks™ HT platform. Using the IonAnalysis software interface, the instrument can be programmed to add compounds to the 384-well PatchPlate™ from either a 96-well compound plate or, alternatively, 1 “quad-

Table 1. Typical Assay Success Rates

<table>
<thead>
<tr>
<th>Success Factor</th>
<th>Dropouts</th>
<th>Total Remaining (384 Possible)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>KV1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Priming of well</td>
<td>2</td>
<td>382</td>
<td>99</td>
</tr>
<tr>
<td>Successful seal</td>
<td>40</td>
<td>342</td>
<td>89</td>
</tr>
<tr>
<td>Channel expression</td>
<td>60</td>
<td>282</td>
<td>73</td>
</tr>
<tr>
<td>Preread/postread stability</td>
<td>20</td>
<td>262</td>
<td>68</td>
</tr>
<tr>
<td>Na III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Priming of well</td>
<td>2</td>
<td>382</td>
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<tr>
<td>Successful seal</td>
<td>20</td>
<td>362</td>
<td>94</td>
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<tr>
<td>Channel expression</td>
<td>20</td>
<td>342</td>
<td>89</td>
</tr>
<tr>
<td>Preread/postread stability</td>
<td>30</td>
<td>312</td>
<td>81</td>
</tr>
</tbody>
</table>

a. Stability criterion: postread within 20% of preread.

FIG. 6. Comparative biophysics of CHO-hNaV1.3 Na+ currents, as measured by conventional patch clamp electrophysiology and Ionworks™ HT. (A) Patch clamp and (B) Ionworks™ HT families of currents obtained using the voltage pulse protocol displayed at the foot of the figure. The horizontal and vertical calibration bars are 1 ms and 300 pA, respectively. (C) The peak current-voltage relationship for the 2 cells shown in (A) and (B).
rant” of a 384-well compound plate. This ensures that each compound gets added to “4” redundant PatchPlate™ wells. As such, depending on the overall success rate of the assay, this results in multiple pharmacological measurements for most compounds. This level of redundancy not only improves the statistical validity of each point, but in a screening application, it also helps to ensure that every test compound is tested successfully in at least 1 PatchPlate™ well. As an example, given an average success rate of 75% and assuming nonsystematic, random failures, the probability that a test compound will not provide a valid physiological measurement for a given assay is (1-0.75)^4 or approximately 1 in 256 compound wells. If this failure rate is unacceptable for a given assay, then the compound plate can be run twice, yielding a failure rate of (1-0.75)^8 or approximately 1 in 65,536 wells.

Assay stability

Measurement stability is a key factor in the ability of the IonWorks™ HT technology to make accurate and precise pharmacological determinations. Figure 7 illustrates the reproducibility/stability of the Kv1.5 channel. The scatter plot represents the output from a typical preread, compound addition, and postread experimental protocol using Kv1.5 CHO cells. In this particular experiment, the compound added to each well was phosphate-buffered saline solution representing inactive “negative” controls. Panel 7A shows the ratio of the peak current amplitudes (I) before (pre) and after (post) buffer addition to the well. Each symbol represents data from a single cell recorded from the same PatchPlate™ during a 35-min run. The distribution of this \(|/|pre\) stability parameter is shown in (B), where cells were assigned to bins of 0.10 size, and the number of cells in each bin is plotted against \(|/|pre\). The fitted line is the Gaussian distribution function, with a mean value of 1.02 and standard deviation of 0.09.
the compounds used in these experiments produced a full block, the variability of the positive controls was negligible in comparison to the negative controls.

**Data processing**

As shown previously, the IonWorks™ HT platform generates high-resolution time traces comparable to a classical patch clamp system. However, due to the quantity of data generated, the ability to reduce the raw traces into pharmacologically relevant values online is essential. In general, the GUI software allows the user to view and/or export raw data or, through specially defined processing “metrics,” view and/or export processed data. An example of a data metric might be the reduction of an entire sodium current trace to a simple number (e.g., the peak inward current value for that trace). The software also has the ability to make comparisons between different (but related) data metrics, for example, the ratio of inward sodium current before compound addition relative to postcompound. This latter feature enables the system to act as a 96-well screening device, monitoring parameters such as compound inhibition and reporting the response of positive and negative control wells much like a plate-based assay.

As an example, Figure 8 display results from an experiment as reported from the GUI. The compound plate view, as shown in Figure 8A, allows the user to view and summarize the experimental results using previously defined data metrics. In this particular example, wells that showed an inhibition of measured current (postcompound relative to precompound) of greater than 40% are highlighted in yellow on the 96-well plate map (Fig. 8A, upper right-hand corner). Also shown on the 96-well compound plate grid is the number of PatchPlate™ wells, which yielded successful physiological measurements for each compound (maximum 4). In the upper left-hand corner of Figure 8A, a numerical data metric representing the average measured current according to the defined metric is displayed for both the precompound and postcompound recording for the cursor-selected well. Figure 8B is a “drill-down” view of the individual precompound (red) and postcompound (green) current recordings for the selected well (C4), allowing for a more rigorous inspection of the underlying electrophysiological data. Together, these tools provide commonly accepted plate-based utilities such as “hit identification” and “control well inspection” in conjunction with user-selected visualization of high-resolution electrophysiological recordings.

**Pharmacology**

To demonstrate the performance of the IonWorks™ HT platform in measuring accurate and reproducible concentration-response curves, one 96-well compound plate was prepared with the hKv1.5 channel blocker tedisamil.14 The plate was prepared as 8 repeat 10-point concentration-response curves to tedisamil (A1-A10, B1-B10, etc.) with columns 11 and 12 as maximum block and vehicle controls, respectively. The purpose of this experiment was to determine if the system could define the IC\textsubscript{50} value for tedisamil for each respective row of the compound plate (i.e., 8 IC\textsubscript{50} estimates per PatchPlate™). Based on the redundancy built into the system, each compound well would be added to 4 wells of the PatchPlate™, providing the potential for 40 measurements per individual 10-point concentration- response curve. An individual measurement point was collected by measuring the peak outward current for a single depolarization step from –70 mV to +50 mV (control), adding tedisamil from the compound plate using the integrated pipettor and then measuring the outward current again. Figure 9 represents the compiled results from this evaluation of intraplate variation, showing the raw data expressed as the ratio of the postdrug to predrug peak current amplitude. The pIC\textsubscript{50} values for tedisamil ranged from 4.86 to 5.02 (mean ± SEM, 4.92 ± 0.05) and the slope values from 0.87 to 4.15 (mean ± SEM, 1.82 ± 1.04). A similar protocol was followed to obtain data for TTX inhibition of hNav1.3 channel currents (data not shown). In this case, the pIC\textsubscript{50} ranged from 8.49 to 8.70 (mean ± SEM, 8.56 ± 0.03) and the slope values from 0.94 to 1.47 (mean ± SEM, 1.26 ± 0.06). In both cases, the number of “qualifying” cells for each IC\textsubscript{50} curve (i.e., acceptable priming, seals, peak currents > 400 pA) ranged from 30 to 38 and yielded curve fits with χ\textsuperscript{2} values of < 0.05. Calculation of Z' values using data from the high (vehicle) and low (highest drug concentration) columns (maximum 32 cells each) yielded values of 0.40 to 0.66 (< 2-point removal from each column).

To assess the performance of the instrument in determining IC\textsubscript{50} values for a wider range of compounds in a single plate, we constructed 10-point concentration-response curves to different Na\textsuperscript+ channel blockers (tetrodotoxin, lidocaine, phenytoin, carbamazepine, veratridine, amitryptiline, fluoxetine and verapramil) (see Fig. 10). The rank order of potency and absolute pIC\textsubscript{50} values are in close agreement to previously published data (where available) for native and recombinant Na\textsuperscript+ channels.12,15-17 A similar outcome was obtained from an analogous experiment with the hKv1.5 cell line. Results from this experiment (data not shown) yielded the following pIC\textsubscript{50}: Ni\textsuperscript{2+} (3.18), Zn\textsuperscript{2+} (2.67), 4-aminopyridine (3.39), fluoxetine (4.46), amitryptiline (4.04), lidocaine (2.54), and tedisamil (4.24). These values compare favorably with reported pIC\textsubscript{50}.14-21 In both experiments, pIC\textsubscript{50} values were readily obtained from each plate, confirming that the 4 PatchPlate™ wells per compound concentration are adequate to define precise pharmacology. As a measure of interplate variability, the same compound plates were screened on a second occasion on different PatchPlates™. The IC\textsubscript{50} values that were obtained correlated extremely well with those from the first determination (R\textsuperscript{2} value = 0.99 for both hNaV1.3 and hKv1.5) (see Fig. 11).

Because of the control over voltage and the ability to measure the ionic currents at high temporal bandwidth, real-time electrophysiology measurements can provide important information about the mechanism(s) by which drugs interact with ion channels. The same voltage control and high temporal resolution of the IonWorks™ HT device enable such mechanism of action...
FIG. 8. (A) Compound plate view, as presented by the IonAnalysis software. In this example, highlighted wells are those that showed a greater than 40% inhibition of the defined current metric after compound addition. Data shown are for an experiment involving hKv1.5, where a 10-point concentration-response curve was generated for 8 different compounds. (B) Individual PatchPlate™ recordings (precompound and postcompound) corresponding to compound plate well C4, which contained 37 µM tedisamil. Note that each compound is added to 4 individual PatchPlate™ wells.
studies to also be conducted but with a much higher throughput. As an example, 2 compounds with distinctly different mechanisms of action were exposed to hKv1.5 channels, as illustrated in Figure 12A. The time-dependent blocking effect of tedisamil (Fig. 12-A1) on hKv1.5 channels, attributed to the drug having to access the open pore of the channel (open-channel block), can be readily distinguished from the time-independent (closed-state) block with Ni²⁺, shown in Figure 12-A2. Using the IonAnalysis software, 2 analysis metrics can be defined: the first captures the drug effect on the current during the early part of the pulse (0-20 ms), and the second captures the drug effect at a later time epoch (150-200 ms). Subtraction of the second metric from the first can be used to quantify the open-channel block. Indeed, with relatively straightforward data manipulation, concentration-response curves for the open-channel block have been easily constructed.

An extension of this use of more than one metric to capture the mechanism of action information is illustrated by studies on the use-dependent block of hNav1.3 channels. Some, but not all, Na⁺ channel blockers preferentially bind to transient states of the channel (i.e., open states, fast inactivation states). Using repeated pulse protocols (e.g., 10 × 20 ms pulses from Vh-90 to V test 0mV at 10Hz), one can accumulate drug access time to these states. Compounds that produce increasing amounts of block via open or inactivation state binding are described as "use dependent." By setting multiple metrics to measure the peak Na⁺ current amplitude at each test pulse, the amount of block can be plotted as a function of the test pulse. With normalization to the amount of block at the first pulse (tonic block), use-dependent and non-use-dependent blockers can easily be distinguished. This is exemplified in Figure 12B1-4 by the action of tetrodotoxin (weakly or nonuse dependent) and amitryptiline (use dependent) on hNav1.3 currents using IonWorks™ HT. Figure 13 demonstrates this same phenomenon of the use-dependent block for 6 different compounds examined from 1 PatchPlate™. Note that the use-dependent effect was

FIG. 9. Repeat concentration-response curves for inhibition of CHO-hKV1.5 K⁺ currents by tedisamil, recorded using IonWorks™ HT. In each case, the abscissa shows the logarithm of the tedisamil concentration (µM) and the ordinate the ratio of the peak current in the presence and absence of drug, determined at 180 to 200 ms after the start of the test pulse (holding potential −70 mV, test potential +50 mV). The pIC₅₀ and slope values for the line of best fit to a 4-parameter logistic equation (see Methods) are shown. All of the data on this figure were obtained from a single PatchPlate™ run in 35 min.
also concentration dependent, and from the data reduction, it is clear that the compounds vary in the rate of accumulated block as well as potency. The similarity of this data set to previous studies, as well as the fact that this single experiment was conducted in < 40 min, illustrates that the IonWorks™ HT can perform complex mechanisms of action analysis on compounds with the resolution of traditional patch clamp methods but with a far greater throughput.

CONCLUSIONS

The power of the patch clamp or any electrophysiological voltage clamp technique lies in the ability to directly measure the fast electrical currents of the ion channel activity while controlling the cell’s membrane potential. The ability to control membrane potential and the subsequent advantage of exploiting the state dependence of certain ion channels targets have already been described.

As the patch clamp is a direct electrical measurement, it offers the additional advantage of stimulating and recording signals with a high degree of temporal resolution, often as fast as 10 kHz. This makes it possible to isolate specific ion channel temporal signatures and hence verify that the measured signal is due to the ion channel of interest. Other ion channel measurement platforms (e.g., those that measure membrane potential) do not kinetically resolve the ion channel event and are subject to other nonspecific changes. In addition, the commonly used fluorescent indicators of membrane potential and intracellular calcium for plate-based measures of ion channel activity are similarly limited. These factors have all combined to make the patch clamp technique the most powerful method to ascertain the function and pharmacology of ion channels. The high-throughput electrophysiology technology developed at Essen was designed to create a technology that couples the high-fidelity measurements made by a standard patch clamp device with a greater than 100-fold increase in throughput or
capacity. Elimination of the manual micromanipulation associated with the standard techniques has also produced a system that requires less skill to operate. The utility has been illustrated by the biophysical and pharmacological characterization of hKv1.5 and hNav1.3 channels expressed in CHO cells. Furthermore, the capability of the instrument to generate precise and consistent concentration-response relationships with the added feature of detection and quantitation of state-dependent and use-dependent drug action is demonstrated.

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REFERENCES

FIG. 12. Detection of state- and use-dependent drug action using IonWorks™ HT. (A) Superimposed hKV1.5 K+ currents in the absence (control) and presence of tedisamil (A1) and Ni²⁺ (A2) obtained using the test protocol shown. The currents in the presence of drug are signaled by the arrow. Note the time-dependent and time-independent block of tedisamil and Ni²⁺, respectively. The horizontal and vertical calibration bars are 40 ms and 1 nA. (B) The use of repeated gating protocols to discriminate use-dependent and non-use-dependent blockers of CHO-hNaV1.3 Na⁺ channels. Na⁺ currents were evoked by a series of 20 pulses to 0 mV of 20-ms duration, 9 Hz from a holding potential of –90 mV. In the absence of drug, there is no decay in the peak amplitude at each pulse with time (B1, B3). In the presence of 1.2 nM TTX (B2), the first pulse is reduced by approximately 50% (vs. B1), but there is no further block with repeated gating. This contrasts markedly to the enhanced blocking effect of 3.7 µM amitriptyline with repeated pulses (B4). The horizontal and vertical calibration bars are 100 ms and 200 pA.


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FIG. 13. Concentration-response curves for 6 different compounds for the use-dependent block of CHO-hNaV1.3 Na+ currents. Data were obtained using the pulse protocol described in Figure 12. Analysis metrics were defined to capture the peak current at each pulse. Data were then normalized to the peak current from the first pulse in the presence of drug to remove the influence of tonic (non-use-dependent) inhibition. The ratio of the current at pulse n to pulse 1 is plotted against the test pulse number. The symbol nomenclature is the same in each panel, in which the highest concentration of drug tested is shown by ● and then subsequent 3-fold dilutions by ▲, ◇, ●, ○, and □, respectively. The highest drug concentrations were as follows: TTX (12 nM), amitryptiline (13 µM), carbamazepine (3.3 mM), lidocaine (3.3 mM), verapamil (111 µM), and fluoxetine (37 µM). All of the data on this figure were obtained from a single PatchPlate™ run in 35 min.