Chapter 9

Recent Advances in Electrophysiology-Based Screening Technology and the Impact upon Ion Channel Discovery Research

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Abstract

Ion channels are recognised as an increasingly tractable class of targets for the discovery and development of new drugs, with a diverse range of ion channel proteins now implicated across a wide variety of disease states and potential therapeutic applications. Whilst the field now ranks as one of the most dynamic fields for drug discovery research, it has historically been regarded by many researchers as a class of proteins associated with numerous technical challenges. Recent advances in our understanding of molecular biology and the increasing acceptance of electrophysiology-based screening methodology mean that ion channels are rapidly progressing towards universal acceptance as worthy and approachable targets for drug discovery. This chapter will outline the commercially available electrophysiology-based screening technologies and give an overview of the range of options for progressing pharmaceutical research and development against this important target class.

Keywords: Ion channel drug discovery, High-throughput electrophysiology, Planar patch-clamp.

1. Introduction

Ion channels are an extremely diverse family of proteins. Located in virtually all cell and tissue types, they make essential contributions towards a wide variety of physiological processes and fundamental homeostatic functions. Without ion channels, the heart would fail to contract or maintain pacing, skeletal muscle would not function, gastrointestinal motility would cease, the immune system would be severely compromised and neurones would fall silent. Ion channels are also implicated in a wide variety of disorders such as epilepsy, cystic fibrosis and cardiac arrhythmias

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making them attractive as targets for therapeutic intervention *(1)*. At the cellular level, ion channels play an essentially simple role, functioning as selective pores controlling the distribution of ions (e.g. Na^+, K^+, Ca^{2+}, Cl^-) across the impermeable lipid bilayer of the cell membrane *(2)*. Acting together with ion pump enzymes, such as Na^+/ K^+-ATPase, ion channels separate ionic charges across the cell membrane, creating both an ionic concentration gradient and an electrical potential difference. Passive movement of ions down this electrochemical gradient is facilitated when ion channels open and, according to cell location and tissue type, modification of electrical potential difference may result in the release of neurotransmitters, firing of neuronal action potentials, muscular contraction, secretion from glandular tissue and activation of an immune response. One particularly important feature of ion channel proteins that must always be taken into consideration when designing screening assays is that they are dynamic structures, able to respond to subtle modifications of the environment surrounding them. This modification may then induce a conformational change, either allowing ions to pass through a central pore or prevent their movement via pore occlusion. Stimuli that can induce a conformational change include a change in potential gradient across the cell membrane, binding of an extracellular ligand or an intracellular messenger, mechanical deformation, change in temperature or a change in proton concentration. Triggering may also be multi-modal, with one example being the transient receptor potential (TRP) family *(3)*, where more than one stimulus may be integrated to elicit a functional response. Although they present unique challenges as drug discovery targets, it is now clearly evident that ion channels have considerable potential to be exploited across a wide variety of therapeutic areas *(1, 4–7)*.

2. Ion Channels as Drug Targets

Therapeutic intervention via modification of ion channel activity is not just an academic concept, since there are well-established prescription drugs on the market that act via ion channel modulation *(7)*. These include the local anaesthetic sodium channel blocker lidocaine (introduced 1948), the sedative chloride channel modulator diazepam (introduced 1963) and the calcium channel inhibitor antihypertensive verapamil (introduced 1982). The majority of such drugs were first marketed between the late 1940s and early 1980s as a result of discovery research where emphasis was placed upon small arrays of compounds screened for in vivo activity, rather than a focused effort to find specific

modulators of a particular channel family or subtype. In the more recent era of high-throughput screening-based drug discovery, where large chemical libraries are generally screened against individual protein targets stably expressed in cell lines, research directed towards the ion channel class of proteins has been highlighted as an undertaking with significant potential pitfalls. The close homology between channel subtypes can mean that finding specific modulators for a particular subtype implicated in a disease state can be very challenging using the target-driven HTS techniques that have become the industry standard approach to discovery research since the 1980s. A further complication is that ion channels do not function as a simple on–off switch; they may pass through a number of conformations or 'states' during the opening or the closing process, changing the availability of potential compound binding sites with each transition. For example, in the case of voltage-gated ion channel blocking compounds, the interaction with the channel may be highly dependent upon the electrical field across the cell membrane (voltage-dependence), it may require the channel to open before the compound is able to bind (open channel block), it may involve an accumulation of block through an imbalance between compound binding and unbinding rate constants (use-dependence) or could occur when the channel is in an inactivated conformation (inactivated state block) *(2)*. Direct clinical evidence that binding to a specific conformation can be advantageous for therapeutic intervention is available if we examine the literature for the iminodibenzyl antiepileptic carbamazepine (Tegretol®), which has been in clinical use since the 1960s. Although not a product of a rational design process to target a particular state of the channel, this compound reduces abnormal action potential discharge patterns and resultant seizure episodes by primarily binding to and stabilising the inactivated configuration of sodium channels *(8)*. The early dihydropyridine calcium channel blockers such as nifedipine through to the more recent 'blockbuster' antihypertensive amlodipine (Norvasc®) also preferentially bind to inactivated channels *(9)*. The knowledge that targeting particular conformational or functional states can be advantageous means that ion channel drug discovery researchers should consider the pharmacological background, the biophysical profile and the likely functional role for a given channel in the disease state before compiling a discovery research strategy. Bringing this knowledge together should facilitate design of a screen biased towards uncovering a particular, and hopefully the most relevant, type of compound interaction with the channel. This could also be advantageous where the actual subtype selectivity may be particularly difficult to achieve, yet where mechanistic selectivity may display an acceptable therapeutic window; for example, identifying use-dependent sodium channel blockers to target rapidly firing sensory neurones for analgesia.

3. Screening for New Drugs

Whilst targeting specific mechanisms of compound interaction is a rational strategy, designing screening methodology for compounds that interact in a specific manner can prove to be a significant challenge. There are no major obstacles to producing evidence regarding interactions of compounds with ion channels when using conventional electrophysiology, a technique enabling very accurate resolution of ion channel activity from individual cells (see below for an overview of methodology). The disadvantages associated with pursuing this approach are the time taken to produce the data, dependence upon highly skilled personnel and the fact that generally only one compound can be examined at any one time. Scaling up to use high-throughput screening methods does not necessarily provide us with a solution. Although such assays enable many more compounds to be examined over a given time period, each assay format involves some element of compromise and may not be the most appropriate starting point for an ion channel research programme. If we consider radioligand binding, an assay technique developed in the 1960s, an appropriate radioactive tracer probe directed towards a single binding site must be prepared and the screen provides results regarding binding to only that site, or one allosterically coupled, with no temporal resolution or accurate voltage control. Hit compounds then require further detailed follow-up using conventional electrophysiology to confirm functional activity. The slow membrane potential-sensitive fluorescence assays, generally based on negatively charged oxonol dyes *(10)*, have relatively good voltage sensitivity and are amenable to scale up to 1536-well format; however, their suboptimal temporal resolution (>1 s) and potential for data being influenced by fluorescence artefacts can reduce confidence in the results generated. They are also unsuitable for fast inactivating sodium channel assays unless an agent such as veratridine is introduced to the assay buffer to artificially prolong channel open time and extend the depolarisation *(11)*. In this screening format, the channel is far removed from its physiological state and the effect veratridine may have on compound or channel interactions is unknown. Again there is no accurate control over cell membrane potential. A number of calcium-sensitive dyes have been developed since they were introduced in the 1980s *(12)* and although they are very useful tools for HTS assay design, they still lack voltage control, their temporal resolution is limited and the readout is secondary to the actual permeation process. Another dye-based approach to resolving membrane potential change is fluorescence resonance energy transfer (FRET). This methodology approaches the timescale required for accurate assessment of ion channel function, with

resolution of potential change within a millisecond timescale. Researchers have also coupled this method with electrical stimulation to measure sodium channel activity under more physiological conditions *(13)*; however, precise regulation of cellular membrane potential is still not available. Both non-radiometric and radiometric efflux have the advantage of assessing activity of compounds via quantification of actual ion permeation through the channel pore. Control of cell membrane potential and temporal resolution are both poor and this functional assessment is limited to ion channel targets with appropriate tracer ions, such as $Li^+$for sodium channels and $Rb^+$for potassium channels *(14)*.

It is the lack of control over cellular membrane potential that significantly compromises data obtained using the above traditional HTS screening assays for ion channel target screening. Additionally, the assay readout generally occurs over a timescale bearing little relevance to the millisecond timescale required to accurately resolve ion channel activity. It would be unreasonable to suggest that the fluorescence- and the efflux-based assay platforms are entirely unsuited to the identification of ion channel modulators though, since they can identify compounds interacting with ion channels, and relatively robust screening assays may be configured to examine large numbers of compounds in a short time period. Fluorescence-based assays combined with electrical stimulation have been shown to facilitate the identification of use-dependent blockers *(13)* or inactivated state blockers *(15, 16)*. However, the lack of accurate voltage control during these assays and inherent compromises of the assay format mean they are still some distance away from the ideal situation and useful compounds may be missed when screening in this type of format. Recent technological advances in electrophysiology-based screening technology have initiated a new era for the field of ion channel drug discovery research. Voltage control, adequate temporal resolution and ability to configure complex recording protocols are now available in electrophysiology-based screening formats with improved throughputs up to 384-well mode. Although still in the relatively early stages of development, these technologies have made a significant impact over a short time period and is set to revolutionise approaches to screening against this challenging target class.

4. Electrophysiology-Based Screening

To fully comprehend the importance and impact of recent technological advances applied to ion channel research, it is important to have some understanding of the most accurate method used for

the study of ion channels, conventional patch-clamp electrophysiology. Pioneered in the early 1980s *(17)*, this versatile method enables precise recording of the current flowing through ion channels contained within the cell membrane, accurately resolving current flow through ion channel pores down to the level of few picoamperes (pA). The method facilitates detailed pharmacological and biophysical characterisation and has been the driving force behind much of our understanding of ion channel function. Whilst immensely powerful, the technique is restricted by the need for complex recording equipment, a requirement for a highly skilled operator and is extremely manually intensive, resulting in throughput typically in the region of 20 data points or 1 EC_{50} or IC_{50} measurement per day. The most significant limitation of this technique is the requirement for a glass microelectrode to be manipulated on to the surface of the target cell to enable formation of a high-resistance seal between the electrode glass and the cell membrane (seal resistance 10^9 Ω or greater). This tight connection, usually referred to as a 'gigaseal', is required to reduce background noise to a level where under the right conditions, amplification can produce recordings with sufficient fidelity to resolve opening of single ion channels. The requirement for significant levels of skilled manipulation and adjustment from the operator during an experiment has limited the scalability and application of this technique. A number of companies recognised this problem and set to work in the late 1990s to design systems aimed at overcoming some of the limitations of the conventional method. Sophion Biosciences launched the Apatchi-1™ automated patch-clamp system in 2001 *(18)*, which used highly sophisticated optics and robotics to mimic the actions performed by a human operator. Originating out of a collaborative effort between NeuroSearch and Pfizer, the system was technically inspired but extremely complex and lacked the consistency or throughput required to give significant advantage over a skilled electrophysiologist using the conventional technique. Around the same time a company called CeNeS Pharmaceuticals in Cambridge, UK, was developing an automated system called the Autopatch™ (or Interface Patch), based on a single inverted glass microelectrode 'blind' patch-clamping cells suspended in a droplet of bathing solution *(19)*. Again, an ingenious approach; but the performance of the equipment was inconsistent, throughput for the first-generation machine offered little if any advantage over the conventional technique and although some pharmaceutical companies evaluated and purchased the system, it was not universally adopted. The technology has since been adopted and refined by Xention Pharmaceuticals, but it is unlikely that the system will become commercially available in the near future. The early automated electrophysiology platforms served to provide evidence that glass microelectrode-based technologies were unsuitable for scale up to provide electrophysiology-based

screening with throughput to compete with 96- or 384-well screening formats. Clearly a new approach was required and this came in the form of planar substrate electrophysiology, a technique that initiated a revolution for both throughput and the contribution that patch-clamp electrophysiology could make to the drug discovery process. This technique replaced the glass microelectrode used in conventional electrophysiology with a flat substrate containing a small hole of around 1–2 μm in diameter (**Fig. 9.1**). Although the actual substrate material varies between individual manufacturers, the recording technique remains fundamentally the same. The important advantage is that a suspension of cells is added to the recording well and formation of a high-resistance seal between a cell and the substrate surrounding a hole is performed automatically by the machine controlling suction force applied behind the planar substrate. Since attraction of cells towards the hole on the substrate is placed under software control, there is no need for complex manipulation equipment and crucially, user intervention. By removing the once laborious and highly skilled process of seal formation, the planar substrate

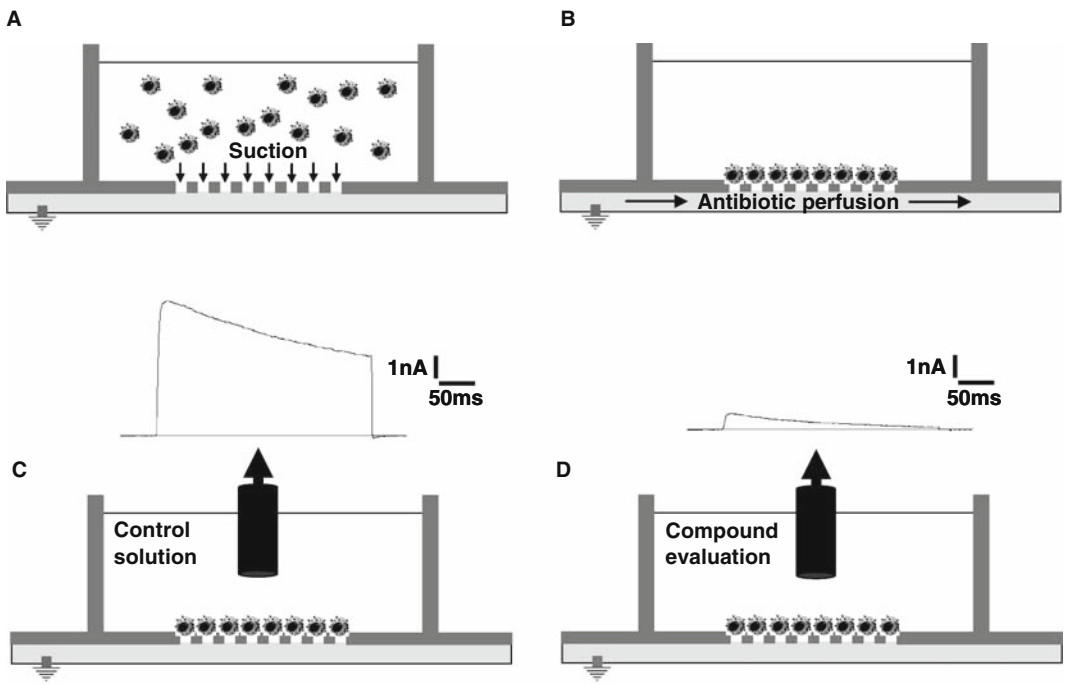

Fig. 9.1. Schematic diagram of a PPC planar substrate; 8 of the 64 available holes are visible in the figure. (**A**) Cells are introduced into the recording well at a defined density (~0.2–1 million cells/ml) and suction is applied from below the planar substrate. (**B**) The suction attracts cells towards the holes and leads to the formation of a seal between the cell and the planar surface. Electrical access is then gained via perfusion of a pore forming antibiotic from below the PatchPlate®. (**C**) Control recordings are taken by moving the headstage electrode into the recording well. (**D**) Following addition of solution containing test compound, a second recording allows evaluation of compound activity.

approach allows all processes following preparation of the cell suspension to be automated. This is the critical advantage and, combined with equipment configuration and automation enabling multiple experiments to take place in parallel, lifts potential daily throughput by a highly significant margin.

IonWorks® HT, a planar substrate patch-clamp electrophysiology system developed by Essen Instruments, considerably increased the potential throughput for electrophysiology-based assays *(20)*. Molecular Devices subsequently secured the worldwide rights to this system and the first commercially available systems were adopted in 2002. The machine is based on a planar substrate, called a PatchPlate®, comprising 384 individual wells each containing a small central hole. By placing the PatchPlate® over a specialised plenum chamber, application of suction under the plate enables the formation of a seal between individual cells and the substrate. A pore-forming agent, such as the antibiotic amphotericin-B, perfused beneath the PatchPlate® facilitates access and electrical control of the cell interior (perforated patch-clamp technique). Once electrical continuity is achieved, a 48-channel recording head automatically reads the plate over eight sets of 48 wells recorded in parallel. For most cell lines, seal resistance values observed with IonWorks® recordings typically fall below the gigaohm level attained using conventional electrophysiology recording and with no inbuilt compensation for series resistance or capacitance, recording fidelity is lower than that obtained with conventional electrophysiology recordings. However, with no requirement for user intervention other than dissociation and loading of cells into the machine, 100–300 successful compound applications are possible in a recording protocol lasting around an hour. When the machine was launched, many researchers recognised that the slight compromise in voltage control and recording fidelity was more than compensated for by the increased throughput afforded by this machine compared to other electrophysiology-based systems. Although the test protocol voltage steps may deviate slightly from the programmed values according to the quality of recordings in individual wells, the machine enables far greater degree of control over the cell membrane potential and assay conditions than any other screening platform with a similar throughput. With appropriate voltage commands the machine can quickly establish information regarding the mechanism of compound block during evaluation of compounds with levels of accuracy that was not possible with previous screening technology. The main drawback of the first-generation IonWorks® is the success rate of acceptable recordings often falling somewhere between 50 and 80% across the PatchPlate®. To compensate for this, four replicates of each drug concentration are typically applied to allow for failed wells. Although a great step forward, with compound supplies, consumables costs and speed

being important commodities for the pharmaceutical industry, it became clear that a more efficient means of evaluating compounds would be required.

5. Population Patch-Clamp

Quadruplicate compound additions for IonWorks® experiments became unnecessary in 2005 when Molecular Devices launched IonWorks® Quattro™. Rather than a completely redesigned instrument, the IonWorks® Quattro™ can be considered to be an evolution of the original IonWorks® HT platform, with changes to the amplifiers and software to enable two distinct recording modes. The single-hole PatchPlate® consumable associated with the IonWorks® HT is still compatible, but a new configuration termed 'population patch-clamp' (PPC) greatly increases recording reliability *(21)*. This configuration utilises a new planar substrate configuration that contains 64 individual holes per well and individual well recordings are composed of the average signal across the 64 holes. There were two main requirements for successful PPC mode recording: first all the 64 holes in a well need to be 'sealed' either by intact cells or by cell debris to give an average resistance across the well ideally of around 50 MΩ or greater (below the 30 MΩ level, data quality becomes unacceptably compromised due to the low seal resistance). The second requirement is that when signals from all holes on the plate are averaged, a sufficient number of intact cells covering the holes express current to a level that offsets the lack of signal from 'non-expressing' cells or cell debris. With cell lines having appropriate expression levels, PPC mode recording success rates can approach 100% acceptable wells across each screening plate. The impact of this enhanced success rate has meant that electrophysiology-based primary screens against ion channel targets are now becoming more commonplace and acceptable to the pharmaceutical industry (**Fig. 9.2**). Whilst more expensive in terms of consumable costs, such screens can provide adequate levels of throughput with substantially higher quality data output than has been possible using fluorescence, binding or efflux methodology. Data are still slightly compromised by the quality of seal between the cells and the planar substrate; but with consistency of recordings, improved throughput and the range of recording metrics available for analysis, the machine significantly outperforms the other more traditional approaches to voltage-gated ion channel screens. IonWorks® Quattro™ can be utilised for assays ranging between examining a few tens of compounds for IC_{50} potency and rank order profiling, up to larger scale primary screening campaigns

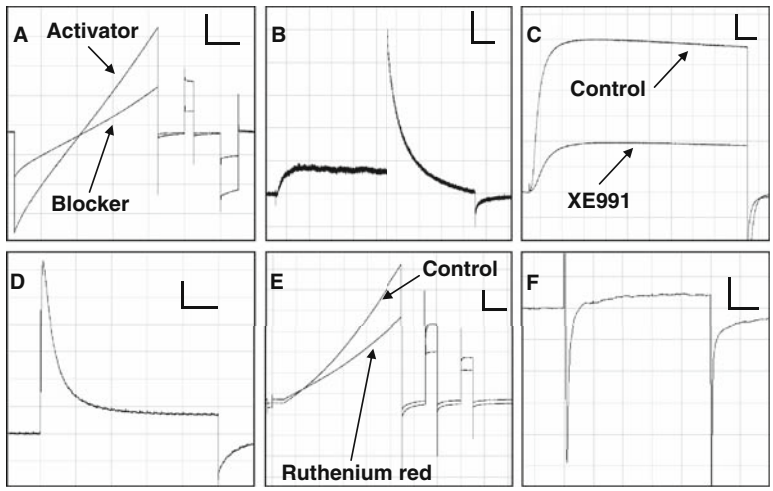

Fig. 9.2. Electrophysiology-based screening is now possible for a wide range of voltage-gated or 'leak' ion channel targets. IonWorks® Quattro™ screening assays configured in the BioFocus DPI research laboratories include **(A)** cystic fibrosis transmembrane conductance regulator (CFTR); **(B)** hERG; **(C)** KCNQ2/3; **(D)** $K_v1.4$; **(E)** TASK3 and **(F)** $Na_v1.7$.

exceeding 50,000 compounds. In a relatively short period of time, the field of ion channel research has moved on considerably, and the utility of the IonWorks® Quattro™ platform for ion channel screens has been recognised by many investigators. Many large pharmaceutical and smaller biotechnology companies have acknowledged the contribution that IonWorks® Quattro™ can make towards progression of their research programmes. The flexibility of assay plate formats and greater level of control over assay conditions mean that electrophysiology-based primary screens are now becoming more commonplace. Since multi-parameter voltage protocols capable of probing the particular states of the ion channel target are also possible, the information content from this type of screen far exceeds what was previously possible. In addition, researchers are beginning to routinely configure assays for ion channels that can present significant technical challenges, for example, calcium-activated potassium channels *(22)*, chloride channels, two-pore domain channels *(23)* and hyperpolarisation-activated cyclic nucleotide-gated channel families *(24)*. Saying that this approach has been universally adopted would be misleading though; the consumable spend, throughput and timeline for this type of screen are considered unacceptable by groups who prefer to use the more traditional fluorescence-based HTS approaches for primary screening and reserve electrophysiology-based methodology for more detailed follow-up studies. The IonWorks® Quattro™ platform still cannot be considered totally satisfactory in the present format. Although the introduction of a 48-channel fluidics

head for compound application has improved throughput compared to the previous 12-channel system, the discontinuous read cycle for the 48-channel electrode head severely limits the throughput of each 384-well plate read. The discontinuous read also means that cells are not voltage-clamped between the control and the drug challenge pulse, which is not an entirely satisfactory situation for an electrophysiology-based assay, although a lengthy pre-read holding period can help to compensate for this. Discontinuous read also means the machine has restricted utility for fast ligand-gated ion channel targets. An improved system with all 384 wells reading simultaneously combined with concurrent compound application would be a partial solution to the current recording limitations and it will be interesting to see whether the technical and financial challenges can be overcome to produce such an improved instrument. The recent launch of the Sophion

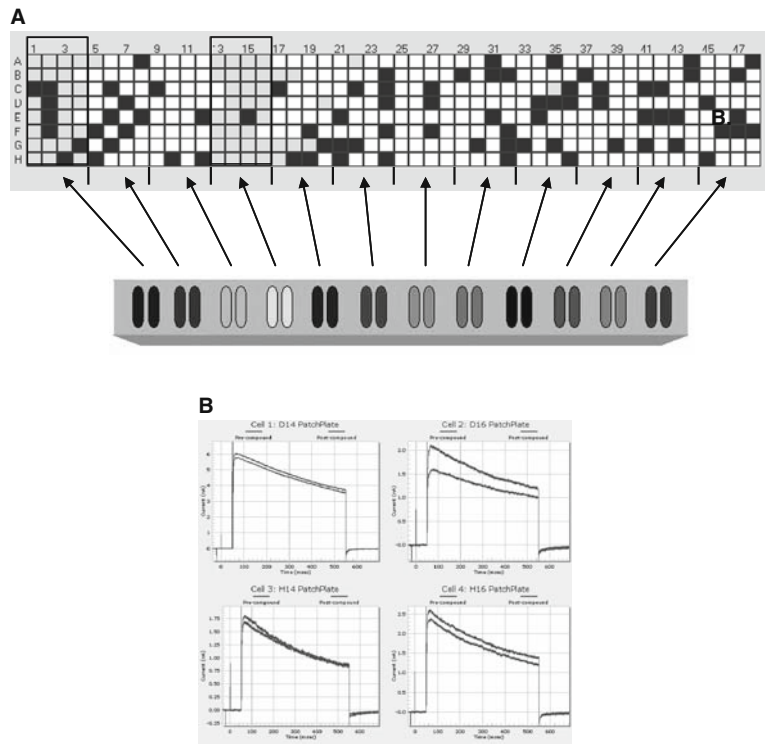

Fig. 9.3. IonWorks® Quattro™ enables rapid functional assessment of putative clonal cell lines. **(A)** Twelve putative clones are examined using a special cell boat that enables 32 individual cells from each clone to be examined for functional expression on one PatchPlate™. Filter metrics are set to show expression above a defined threshold. In this instance two clones are highlighted on the PatchPlate view where functional expression exceeded 1 nA for a significant majority of the cells evaluated. **(B)** Four example current traces from a clone selection exercise; current amplitude, inactivation characteristics and stability over two read cycles are all evident and can be rapidly evaluated.

Qpatch™ HTX illustrates the attractiveness of planar substrates containing multiple holes. This instrument is a revision of the 48 channel QPatch™ HT and utilises and QPlate consumable containing multipleholes in each recording well to increase recording success rates. The facility for continuous recording and compound application has clear benefits for study of challenging ligand-gated ion channels and the machine offers significant potential for improving throughput in this area of ion channel research.

With IonWorks® Quattro™ PPC mode recording showing such high success rates, the utility of single-hole PatchPlates™ could be questioned. However, the single-hole substrate is particularly well suited to rapid screening for functional current expression during cell line generation programmes. With a typical throughput of up to 120 clones in a day and data generated for 32 individual recordings per clone, this is a powerful technique for rapid progression of stable cell line generation (**Fig. 9.3**). Construction of high-quality clonal cell lines is a vitally important part of drug discovery and fast decision making for the progression of putative clones towards validation is a real benefit. The ability to select a particular cell line according to the expression level and seal quality can significantly impact timeline and cost for the subsequent screening campaign. There is also an added benefit that clones identified for functional expression on IonWorks® generally perform well for conventional and other electrophysiology formats. Single-hole PatchPlate® recording is also useful for assay validation and optimisation before transfer to PPC mode.

Although not without technical limitations, IonWorks® Quattro™ firmly led the medium throughput electrophysiology-based screening field for voltage-gated ion channel targets in the early stages of 2008. The diverse experimental applications and flexible recording formats mean it is likely to remain an invaluable tool for ion channel discovery research for some years to come.

6. Giga Seal Quality Automated Electrophysiology

Planar patch-clamp systems that give rise to higher fidelity data recordings include the PatchXpress® 7000A from Molecular Devices (introduced in 2002) *(25)*, the QPatch™ 16 from Sophion Biosciences (introduced in 2003) *(18, 26)* and the NPC-16 Patchliner from Nanion Technologies (introduced in 2006) *(27)*. All three machines operate via a 16-well planar substrate recording chip and subsequently have considerably lower throughput than IonWorks® Quattro™. However, they have the advantage of active monitoring of individual recordings with software control over decisions regarding actions such as the

application of suction to maintain recording quality or the perfusion of the next compound. Data quality is comparable to conventional electrophysiology, with the advantage that the instruments can potentially record from up to 16 individual cells per recording session, although recording success rates more typically lie between 6 and 10 completed experiments. This boost in throughput over the conventional technique means the instruments are generally used for compound follow-up after a primary screen, safety pharmacology such as hERG profiling and pharmacological validation of clonal cell lines. PatchXpress® and QPatch™ 16 are true 16-channel systems able to record in parallel from all wells simultaneously and independently. Both machines have now been adopted by a wide range of pharmaceutical companies and contract research organisations. QPatch™ has a more complex planar substrate arrangement than PatchXpress®, with each QPlate incorporating a compound reservoir, a laminar flow channel driven by a passive capillary action and a waste reservoir. This enables small volumes of compound to be applied to a cell with very fast solution exchange, making the system well suited to the study of both fast ligand-gated and voltage-gated ion channels. Due to the presence of an onboard cell handling station, which is able to maintain cells in good condition for up to 4 hours, extended periods of unattended use are possible with this system. Whilst PatchXpress® employs a less-complex drug application system, via simultaneous compound perfusion and aspiration into a simple well structure, it is also able to achieve rapid solution exchange rates consistent with recording high-quality ligand- and voltage-gated responses. PatchXpress® does not have an integrated cell maintenance station and therefore does require more user input than QPatch™. Potential data throughput is similar for both machines; the liquid handling system of the QPatch™ instruments can be configured with four or eight pipettes as opposed to the single pipettor specified on the PatchXpress® though, which can help to speed up QPatch™ experiment times especially ligand-gated experiments where the pipettors are in great demand. The most significant difference between PatchXpress® and QPatch™ 16 is scalability. PatchXpress® took advantage of the Axon Instruments MultiClamp amplifiers normally used for conventional electrophysiology and, although very high-precision state-of-the-art recording units, their physical size means there is little scope for expansion of the number of recording channels within the PatchXpress® instrument. Rather than taking advantage of existing technology, QPatch™ was designed from the outset to be a scalable system. By using miniaturised printed circuit board amplifiers (designed by Alembic Instruments) that contain 16 separate amplifier circuits per board, significant expansion of recording capability has been made available within the QPatch™ chassis. The QPlate consumable was also designed to offer capacity

upgrade and with the recent introduction of a new 48-channel QPatch™ HT chip, existing users with a QPatch™ 16 may now upgrade their existing system or new users can purchase the QPatch™ HT recording system. Both the upgrade and the QPatch™ HT have a significantly enhanced throughput compared to the other commercially available giga seal quality systems. Whether the increased capital outlay and cost of consumables will hinder widespread adoption of this system remains to be seen, although the cost of consumables has not proven to be a particularly significant barrier to companies adopting other high-throughput electrophysiology systems in their laboratories.

An automated system that is probably less widely recognised is the Patchliner© from Nanion *(27)*. This benchtop machine, based on HEKA EPC-10 amplifiers and a Tecan liquid handling robot, has a significantly smaller footprint than either the QPatch™ or the PatchXpress®. Patchliner© uses a 16-channel planar substrate and is able to produce high-quality giga seal recordings in an automated format. Patchliner© can be configured to run between 2 and 8 channels in parallel with unattended use for periods of up to 4 hours. It also has the advantage of being able to rapidly exchange internal and external recording solutions through a specially designed glass chip, facilitating detailed biophysical and pharmacological analysis of the compound mechanism of action. The flexibility of this system means it has been adopted by both academic and industry laboratories and it will be interesting to see whether the instrument, and the recently launched 96 channel SynchroPatch® 96, gain wider acceptance over the next few years.

Another automated system based on HEKA amplifiers and a Tecan liquid handling robot is the Flyscreen® 8500 from Flyion *(28)*. This screening system with options for three or six recording channels has been developed around a novel means of recording from cells. Rather than pursuing the planar substrate route, the device records from cells sealed within a 'FlipTip' microelectrode structure. The single-use glass consumables are similar to those used in conventional electrophysiology and high-resistance seals are formed by simply flushing a suspension of cells towards the tip. This imaginative approach to obtaining a giga seal quality recording not only facilitates automated software-controlled recording, but also negates the requirement for expensive micromanipulators or planar chip consumables. Since the high-resistance giga seal is formed within the tip of the glass electrode, recordings are less susceptible to failure than conventional electrophysiology recordings due to vibration or other external forces. The intracellular face of the microelectrode is housed within a plastic insert, which contains recording solution, and facilitates the application of suction to gain electrical access to the cell interior. Since compounds are applied inside the microelectrode structure, there have been some concerns regarding the speed of solution exchange within

the tip of the FlipTip consumable and the potential for reduced compound potency due to clumps of cells restricting compound access. The concerns have been addressed via the introduction of a new microforge-based electrode manufacturing system, which significantly improves compound access for this type of recording. Microelectrodes are fabricated using an automated feedback-controlled, pressure-polishing microforge employing CCD-based tip recognition software. This system fabricates electrodes with both a very short tip section and a significantly enlarged shank region for perfusion of solutions. Using these electrodes, solution exchange rates as fast as 50 ms can be achieved and recordings of fast ligand-gated responses have been demonstrated.

7. Single-Channel Planar Patch-Clamp Systems

Lower throughput giga seal quality semiautomated planar substrate electrophysiology systems are also now available. These single-channel systems enable researchers to perform patch-clamp electrophysiology recordings without the need for extensive periods of training. Since the new single-channel systems are bench mounted, there is a considerable space saving compared to a conventional electrophysiology system, which requires at least 1.5 m^2 of floor space for an average sized vibration isolation table and amplifier. The time taken to set up and be ready to record is a matter of minutes with the new systems, in stark contrast to a conventional electrophysiology rig, which requires practical skills and at least half a day of assembly time during initial set-up, followed by an inevitable period of adjustment to both perfusion and electrical connections to achieve stable recordings with low levels of extraneous electrical noise. The Port-a-Patch© system from Nanion miniaturises patch-clamp electrophysiology recording apparatus down to a total benchtop footprint of around 0.18 m^2 including the amplifier *(27)*. Using essentially the same planar technology as the Patchliner©, but reduced to fit a single-channel format, it enables scientists to make high-quality patch-clamp measurements from a wide range of both cultured and primary cell types. The small size, ease of use and versatility to exchange both intracellular and extracellular solutions mean this instrument is likely becoming increasingly popular in both academic and industry electrophysiology laboratories. In addition, the Port-a-Patch© can utilise either the industry standard Axon or HEKA amplifiers with their respective software and therefore be incorporated quickly into an existing electrophysiology group.

The PatchBox from Flyion also offers a benchtop single-channel patch-clamp recording system and uses the same principle as the Flyscreen® 8500, where the recording occurs inside a

microelectrode structure rather than patching on a planar substrate. With an amplifier and a PatchBox footprint of around 0.25 m^2, this system uses electrodes fabricated using a conventional microelectrode puller. As with the Nanion Port-a-Patch, the system is compatible with HEKA amplification. Whilst this approach may be more suitable for experienced electrophysiologists, it does provide a level of experimental flexibility and consumable cost effectiveness that may be lacking in systems with prefabricated substrates.

8. Single-Channel Non-Planar Patch-Clamp Systems

The Cellectricon Dynaflow® system, first launched in 2003, is a giga seal quality recording system that uses a prefabricated chip with inbuilt laminar flow channels to facilitate rapid solution exchange around a single cell suspended at the end of a conventional patch pipette *(29)*. Cell preparation for this technique therefore involves a step to dissociate cells, enabling a cell to be patched, subsequently detached from the substrate and placed in the vicinity of solutions perfused under laminar flow conditions. These steps require the operator to be trained and experienced with conventional electrophysiology techniques. To facilitate accurate switching between different solutions, the Dynaflow® chip is housed on a modified microscope stage, which allows the position of the laminar flow channels to be accurately controlled by a software-driven motorised scan stage. Solution exchange is then enabled by placing the cell in one laminar stream with rapid movement of the stage switching the cell between laminar streams containing control buffer or test solutions. Whilst the technique is applicable to rapid screening of compounds for both voltage-gated and ligand-gated ion channels, the system is particularly suited to fast desensitising ligand-gated channels due to the very rapid solution exchange possible between laminar flow channels. Three different Dynaflow® chips are now available; accommodating 8, 16 or 48 different compounds, facilitating testing of multiple compounds per recording. Early reports of the system identified issues of certain compounds being absorbed into the structure of the chip, resulting in inaccurate potency reporting; however, the chips have since been modified to minimise compound absorption and the manufacturers claim to have rectified this issue. Cellectricon have recently announced the launch of an automated high-throughput electrophysiological instrument based around their micro-fluidics expertise. The instrument is a 96 channel system with the capacity to make the rapid solution changes necessary for the screening of fast desensitising ligand-gated ion channels and will be available in late 2009.

9. Oocyte-Based Automated Voltage Clamp

Xenopus oocytes have been used as a model system for the expression and study of ion channels and transporter proteins for many years. Their robust nature and ability to rapidly express protein following injection of messenger RNA has meant that they have often been utilised for study of both voltage- and ligand-gated channels. Automated systems such as the eight-channel OpusX press® 6000A voltage clamp system *(30)* and the 96-well format Robocyte from Multichannel Systems *(31)* offer recording in a format requiring minimal user training and/or intervention. The Robocyte has the advantage of unattended overnight injection and recording of responses, whilst the OpusXpress® records simultaneous responses in parallel to boost throughput. Both machines offer the capability of investigating ion channel targets without having to create a stable cell line, although some caution is advised when generating data from oocyte systems due to the non-mammalian cell background and the potential for compound partitioning or absorption into the yolk.

10. Impact of the Technological Advances

The early years of the 21st century have seen a dramatic growth in the number of commercially available electrophysiology-based technologies able to facilitate progression of both pharmaceutical and academic research campaigns. Among the technological advances outlined above, planar substrate recording must rank as the most exciting development to have emerged. Prioritisation of compounds can now occur over a timescale that would not have been possible a decade ago. Planar patch now enables rapid profiling of high-value compounds in secondary assays such as hERG with giga seal quality recording, all the way up to medium-throughput electrophysiology-based screening of libraries up to ~100,000 compounds. Crucially, electrophysiology-based primary screens recording in PPC mode can now quickly provide information regarding both level of compound activity and putative mechanism of action in a screening format with unrivalled control over the assay conditions. The technology is still relatively new and there is significant scope for refinement of most of the available systems. IonWorks® Quattro™ in particular being compromised by the discontinuous compound addition and recording phase, which slows down throughput, limits voltage control to defined periods during the assay and makes assay development for

fast ligand-gated ion channels either impossible or particularly challenging. Whilst some researchers have begun to configure IonWorks® Quattro™ assays to address fast ligand-gated channels such as $GABA_A$, continuous voltage clamping, compound addition and reading would be a more satisfactory and versatile solution. All planar systems also suffer from one further disadvantage, the cost of the consumable. This is a particular burden for research budgets and deters some research teams from fully exploiting the potential of the new technology.

Whilst the new electrophysiology-based technologies have probably had less impact for academic researchers than for groups from within the pharmaceutical industry, there are signs that this will change in the next few years. The main barriers to entry in this market for grant-funded university groups are the initial cost of the equipment, high consumables costs and lack of real need to generate many thousands of data points per week. The lower throughput giga seal quality 1- to 16-channel automated machines are most likely to be the first systems adopted, facilitating rapid high-quality pharmacological profiling experiments and allowing molecular biologists to evaluate new ion channel clones without a protracted period of training. Some university groups such as the Faculty of Biological Sciences in Leeds (UK) have already taken the first step *(32)* and it will be interesting to see where the new technologies will begin to impact research in the academic environment over the next few years. The advantages to the pharmaceutical industry are much clearer; with an ever increasing demand for novel drugs to replace those nearing the end of their patent life, the wide range of ion channel targets now implicated across diverse therapeutic areas and the potential to secure vast amounts of future revenue, significant capital and consumable resource investment becomes much easier to justify. How automated electro physiology resource is used within each company will be determined by a combination of whether managements are willing to move back towards ion channel targets, whether they are willing to move away from the tried and trusted traditional HTS approaches and their ability to absorb the increased consumables spend that accompanies a move into electrophysiology-based screening. Many large pharmaceutical companies may still be smarting from their first move into ion channels, where significant amounts of money were invested for comparatively little return on the investment. Others will see this opportunity as the way to revisit and move forward earlier campaigns or start afresh with new insight and suitable screening tools. For the smaller specialist biotechnology companies it is likely that electrophysiology-based screening will be universally adopted over a faster timescale, with companies taking advantage of the technology to rapidly develop expertise within their own niche area. Unencumbered by a corporate bias towards particular experimental approaches, perhaps more freedom to

explore novel approaches and a need for rapid data generation to secure funding, this sector may benefit significantly from the additional data available when adopting an electrophysiology-based screening approach.

It is clear that a number of strategic options are now available to pharmaceutical industry researchers wishing to investigate ion channel targets (**Fig. 9.4**). The most conservative approach is to identify and characterise hits from a large compound library ($\geq$100,000 compounds) using the traditional fluorescence-based or other HTS methodologies and then progress only a small subset of hits meeting potency and selectivity criteria towards giga seal quality electrophysiology-based validation. This approach is likely to be adopted by larger pharmaceutical companies who still wish to screen large libraries using an assay format consistent with previous screening campaigns. A slightly modified version of this approach is again to screen a large ($\geq$100,000) library using fluorescence

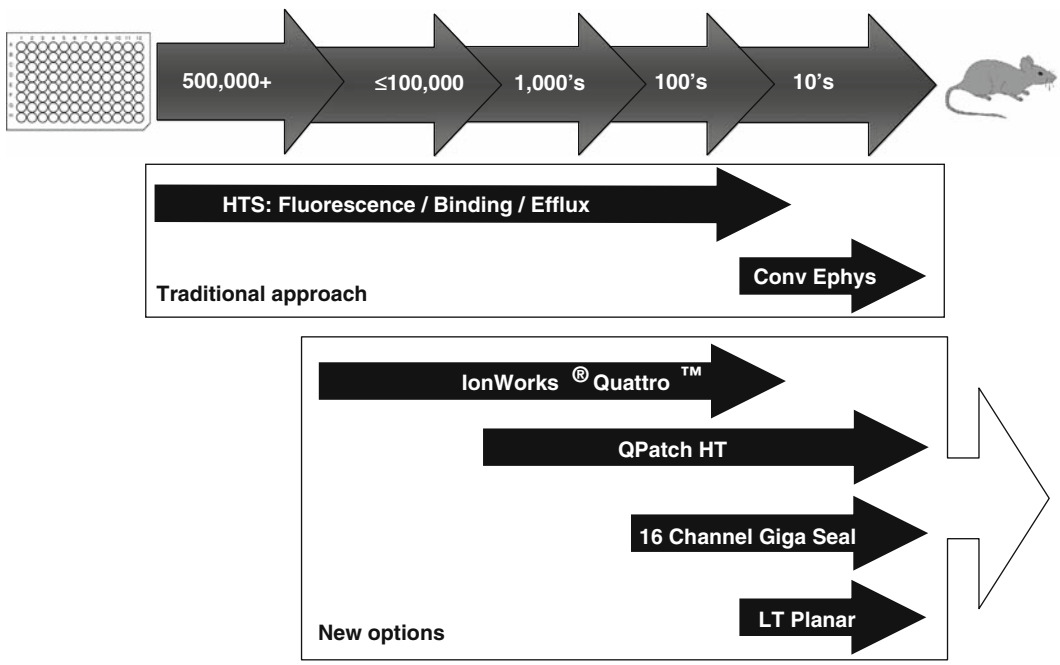

Fig. 9.4. A number of strategic approaches for progressing ion channel discovery research are now available. Whilst the traditional HTS-biased approach involves emphasis on standard plate-based assays with a relatively small contribution from conventional electrophysiology (Conv Ephys), the new commercially available electrophysiology formats give many options for the point where electrophysiology makes a contribution. This may still involve a standard HTS campaign, but could now involve IonWorks® Quattro™-based primary screening of a relatively large compound collection. Screen follow-up can now include profiling of hundreds to thousands of compounds using IonWorks® Quattro™ or the multi-channel giga seal quality systems such as PatchXpress®, QPatch™ or Patchliner©. Finally detailed assessment of compounds can occur using either low-throughput planar substrate systems (LT planar) or the conventional technique.

techniques and then move directly to PPC and/or giga seal quality automated electrophysiology to characterise and progress the confirmed hits. Both of these approaches may be compromised by the use of the HTS methodologies in the initial phase, but they do have the advantage of speed for the primary screen and ability to examine very large compound libraries. Another approach that is starting to gain favour amongst a number of industry researchers is to perform the primary screen using PPC planar substrate electrophysiology. Here, either small focused compound collections of a few thousand compounds or even compound collections ($\leq$100,000) representing the diversity space from a larger collection are examined for activity in the electrophysiology assay, the rationale behind this approach being that the larger consumable costs are offset by the increased likelihood of finding high-quality hits via the additional control of the assay conditions and the ability to develop relatively complex multi-parameter screening protocols. Significant further value can be added to the electrophysiology-based screening approach via input from experienced chemists and use of in silico techniques in the initial stages to design a focused library directed towards the target of interest. Large corporations may elect to compile such a focused or directed compound collection via selection from within their large diverse compound library, whilst smaller companies may opt to use specialist chemical providers such as Asinex, Chemdiv, Albany Molecular Research or BioFocus DPI to purchase an off-the-shelf ion channel-directed collection or to create a bespoke focused library.

It would be unfortunate if the more traditional conventional patch-clamp techniques are written off prematurely though; the quality of data and sheer versatility of recording configurations remain as a significant strength that should not be overlooked. Indeed, in the short term the introduction of planar substrate electrophysiology will most likely benefit the conventional technique, with the planar technology platforms able to act as fast filters allowing valuable conventional electrophysiology resource to be reserved for complex biophysical and pharmacological analysis. It would be too easy to forget that the conventional technique has produced a generation of skilled electrophysiologists who have great technical ability and an in-depth understanding of the challenges associated with the investigation of ion channel proteins. This level of understanding is critical for effective prosecution of a discovery research programme directed against ion channels and needs to be maintained, regardless of the recording format being used.

It is difficult to imagine that the field of automated electrophysiology will continue to evolve at the rate we have recently experienced. The technology and techniques are clearly robust and will no doubt be refined further to address more challenging

targets and enable greater throughput and recording fidelity. Although most ion channel specialists believe we are now armed with powerful tools to facilitate progression of screening campaigns towards the eventual goal of a therapeutically useful drug, the crucial test will be whether this technology and associated expense actually produce that additional edge over the previous technologies. Clearly this debate will not be resolved for some time, but with the renewed appetite for companies to address ion channel targets and the quality and breadth of assays now being configured by many industry groups, it is difficult to imagine that within the next decade we will not see evidence for automated electrophysiology accelerating the identification of new ion channel modulators.

References

1. Bernard, G., Shevell, M.I. (2008) Channelopathies: a review. *Pediatr. Neurol.* **38**(2), 73–85.
2. Hille, B. (2001) *Ion Channels of Excitable Membranes*. Sinauer Associates, Inc.
3. Clapham, D. (2006) An introduction to TRP channels. *Ann. Rev. Physiol.* **68**, 619–647.
4. Gosling, M., Poll, C., Li, S. (2005) TRP channels in airway smooth muscle as therapeutic targets. *Nayun-Schmiedebergs Arch. Pharmacol.* **371**, 277–284.
5. Xie, M., Holmqvist, M.H., Hsia, A.Y. (2004, April) Ion channel drug discovery expands into new disease areas. *Curr. Drug Discov.*, 31–33.
6. Southan, A., James, I.F., Cronk, D. (2005) Ion channels new opportunities for an established target class. *Drug Discov. World* **6** (3), 17–23.
7. Hogg, D.S., Boden, P., Lawton, G., Kozlowski, R.Z. (2006) Ion channel drug targets unlocking the potential. *Drug Discov. World* 7(3), 83–92.
8. Yang, Y.C., Kuo, C.C. (2005) An inactivation stabilizer of the Na^+channel acts as an opportunistic pore blocker modulated by external Na^+. *J. Gen. Physiol.* **125**(5), 465–481.
9. Koidl, B., Miyawaki, N., Tritthart, H.A. (1997) A novel benzothiazine Ca^{2+}channel antagonist, semotiadil, inhibits cardiac L-type Ca^{2+}currents. *Eur. J. Pharmacol.* **322**(2–3), 243–247.
10. Baxter, D.F., Kirk, M., Garcia, A.F., Raimondi, A., Holmqvist, M.H., Flint, K.K., Bojanic, D., Distefano, P.S., Curtis, R., Xie, Y. (2002) A novel membrane potential-sensitive fluorescent dye improves cell-based assays for ion channels. *J. Biomol. Screen.* 7(1), 79–85.
11. Vickery, R., Amagasu, S., Chang, R., Mai, N., Kaufman, E., Martin, J., Hembrado, J., O'Keefe, M., Gee, C., Marquess, D., Smith., J. (2004) Comparison of the Pharmacological properties of rat NaV1.8 with rat NaV1.2a and human NaV1.5 voltage-gated sodium channel subtypes using a membrane potential sensitive dye and FLIPR. *Receptors and Channels* **10**(1), 11–23.
12. Minta, A., Kao, J.P., Tsien, R.Y. (1989) Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. *J. Biol. Chem.* **264**, 8171–8178.
13. Huang, C.J., Harootunian, A., Maher, M.P., Quan, C., Raj, C.D., McCormack, K., Numann, R., Negulescu, P.A., González, J.E. (2006) Characterization of voltage-gated sodium-channel blockers by electrical stimulation and fluorescence detection of membrane potential. *Nat. Biotechnol.* **24**(4), 415–416.
14. Gill, R., Lee, S.S, Hesketh, J.C., Fedida, D., Rezazadeh, S., Stankovich, L., Liang D. (2003) Flux assays in high throughput screening of ion channels in drug discovery. *Assay Drug Dev. Technol.* **1**(5), 709–717.
15. Liu, C.J., Priest, B.T., Bugianesi, R.M., Dulski, P.M., Felix, J.P., Dick, I.E., Brochu, R.M., Knaus, H.G., Middleton, R.E., Kaczorowski, G.J., Slaughter, R.S., Garcia, M.L., Köhler, M.G. (2006) A high-capacity membrane potential FRET-based assay for NaV1.8 channels. *Assay Drug Dev. Technol.* **4,** 37–48.

16. Kolok, S., Nagy, J., Szombathelyi, Z., Tarnawa, I. (2006) Functional characterization of sodium channel blockers by membrane potential measurements in cerebellar neurons: Prediction of compound preference for the open/inactivated state. *Neurochem. Int.* **49**, 593–604.
17. Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* **391**, 85–100.
18. Asmild, M., Oswald, N., Krzywkowski, K.M., Friis, S., Jacobsen, R.B., Reuter, D., Taboryski, R., Kutchinsky, J., Vestergaard, R.k., Schrøder, R.l., Sørensen, C.B., Bech, M., Korsgaard, M.P.G., Willumsen, N. (2003) Upscaling and automation of electrophysiology: Toward high throughput screening in ion channel drug discovery. *Receptors and Channels* **9**, 49–58.
19. Owen, D., Silverthorne, A. (2002) Channelling drug discovery, current trends in ion channel discovery research. *Drug Discov. World* **3**(2), 48–61.
20. Schroeder, K., Neagle, B., Trezise, D.J., Worley, J. (2003) Ionworks HT: A new high-throughput electrophysiology measurement platform. *J. Biomol. Screen.* **8**(1), 50–64.
21. Finkel, A., Wittel, A., Yang, N., Handran, S., Hughes, J., Costantin, J. (2006) Population patch clamp improves data consistency and success rates in the measurement of ionic currents. *J. Biomol. Screen.* **11**(5), 488–496.
22. John, V.H., Dale, T.J., Hollands, E.C., Chen, M.X., Partington, L., Downie, D.L., Meadows, H.J., Trezise, D.J. (2007) Novel 384-well population patch clamp electrophysiology assays for Ca^{2+}-activated K^{+}channels. *J. Biomol. Screen.* **12**(1), 50–60.
23. Clark, G.S., Todd, D., Liness, S., Maidment, S.A., Dowler, S., Southan, A.P. (2005) Expression and characterisation of a two pore potassium channel in HEK293 cells using different assay platforms. *Proceedings of the British Pharmacological Society*at http://www.pA2online.org/abstracts/Vol3Issue4abst105P.pdf.
24. Lee, Y.T., Vasilyev, D.V., Shan, Q.J., Dunlop, J., Mayer, S., Bowlby, M.R. (2008) Novel pharmacological activity of loperamide and CP-339,818 on human HCN channels characterized with an automated electrophysiology assay. *Eur. J. Pharmacol.* **581**(1–2), 97–104.
25. Tao, H., Santa Ana, D., Guia., A, Huang, M., Ligutti, J., Walker, G., Sithiphong, K., Chan, F., Guoliang, T., Zozulya, Z., Saya, S., Phimmachack, R., Sie, C., Yuan, J., Wu, L., Xu, J., Ghetti, A. (2004) Automated tight seal electrophysiology for assessing the potential hERG liability of pharmaceutical compounds. *Assay Drug Dev. Technol.* **2**(5), 497–506.
26. Kutchinsky, J., Friis, S., Asmild, M., Taboryski, R., Pedersen, S., Vestergaard, R.K., Jacobsen, R.B., Krzywkowski, K., Schrøder, R.L., Ljungstrøm, T., Hélix, N., Sørensen, C.B., Bech, M., Willumsen, N.J. (2003) Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. *Assay Drug Dev. Technol.* **1**(5), 685–693.
27. Farre, C., Stoelzle, S., Haarmann, C., George, M., Brüggemann, A., Fertig, N. (2007) Automated ion channel screening: patch clamping made easy. Ion channel drug discovery expands into new disease areas. *Expert Opin. Ther. Targets* **11**(4), 557–565.
28. Lepple-Wienhues, A., Ferlinz, K., Seeger, A., Schäfer, A. (2003) Flip the tip: An automated, high quality, cost-effective patch clamp screen. *Receptors Channels* **9**(1), 13–17.
29. Dunlop, J., Roncarati, R., Jow, B., Bothmann, H., Lock, T., Kowal, D., Bowlby, M., Terstappen, G.C. (2007) In vitro screening strategies for nicotinic receptor ligands. *Biochem. Pharmacol.* **74**(8), 1172–1181.
30. Papke, R.L. (2006) Estimation of both the potency and efficacy of alpha7 nAChR agonists from single-concentration responses. *Life Sci.* **78**(24), 2812–2819.
31. Schnizler, K., Küster, M., Methfessel, C., Fejtl, M. (2003) The robocyte: Automated cDNA/mRNA injection and subsequent TEVC recording on Xenopus oocytes in 96-well microtiter plates. *Receptors Channels* **9**(1), 41–48.
32. Xu, S.-Z., Sukumar, P., Zeng, F., Li, J., Jairaman, A., English, A., Naylor, J., Ciurtin, C., Majeed, Y., Milligan, C.J., Bahnasi, Y.M., Al-Shawaf, E., Porter, K.E., Jiang, L.-H., Emery, P., Sivaprasadarao, A., Beech, D.J. (2008) TRPC channel stimulation by extracellular thioredoxin. *Nature* **451** (7174), 69–73.