Drug discovery technology for ion channels

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Although technologies for ion channel screening have not kept pace with other HTS assays in the past, emerging technologies promise to shift this paradigm in the near future. This was the focus of the 2nd annual *Drug Discovery for Ion Channels* satellite meeting of the Biophysical Society (22 February 2002, San Francisco, CA, USA), sponsored by Axon Instruments. It represented an opportunity for screening technology companies to report their progress made in 2001, to academic and pharmaceutical or biotechnology investigators interested in ion channel drug discovery.

Alan Finkel of Axon Instruments (Union City, CA, USA) set the stage for the meeting by remarking on the explosion of information about ion channel function that has occurred over the past 20 years with the advent of the patch clamp techniques and more recently, the ability to study ion channel function using high-throughput fluorescent screening techniques such as the Fluorescent Imaging Plate Reader (FLIPR; Molecular Devices, Sunnyvale, CA, USA) and the Voltage Ion Plate Reader (VIPR; Aurora Bioscience, San Diego, CA, USA).

However, the excitement at this year's meeting was the fast-paced progress in the automated patch-clamp field that has occurred in the past year. Specifically, up until 2002, planar patch-clamp technologies had yet to be validated because no research group had presented whole-cell recordings from single mammalian cells at the meeting in 2001. By contrast, several groups presented whole-cell data examples at this year's meeting.

Pharmaceutical perspective

Early discussions focused on how some of the current technologies are implemented in the pharmaceutical industry today. Owen McManus from Merck (Rahway, NJ, USA) shared his perspective on ion-channel technologies used in drug discovery. Specifically, he described the use of classical ligand-binding techniques, as well as rubidium efflux (which is useful for assessing K^+ channel function). In addition, he described fluorescent assays on both FLIPR and VIPR platforms using Fluo-4, a Ca^{2+} indicator for studying vanilloid receptor 1 (VR1). He commented that the patch-clamp technique is undoubtedly the best technique for ion channel function because it has the required flexibility and temporal resolution. Furthermore, he stated that for high-throughput patch-clamping to be successful, it will need to have the ability to generate robust, high-quality data using established cell lines at reasonable cost with efficient data handling.

Automated electrophysiology

Axon Instruments launched their automated two-electrode voltage clamp (TEVC) system (OpusXpress™) for *Xenopus* oocytes at the meeting with Cathy Smith-Maxwell highlighting the features of the system. OpusXpress records from eight oocytes in parallel and can run unattended for a total of 24 automated solution changes per oocyte. In addition to Axon Instruments, Michael Fejtl of Multi-Channel Systems (Reutlingen, Germany) presented information about their commercially available TEVC system called Roboocyte. The Roboocyte measures ion channel currents from channels expressed in oocytes using a single recording head containing the voltage recording, current injection and ground electrodes, as well as the perfusion inlet and outlet. The Roboocyte is capable of running unattended and will automatically inject mRNA or cDNA into the oocytes before recording. In contrast to these TEVC systems, Morton Bech of Sophion Bioscience (Ballerup, Denmark) presented information about their Apatchi-1, an automated patch-clamp system capable of generating whole cell data. This system uses a conventional microscope, amplifier, and patch-clamp pipettes.

Planar patch-clamp technologies

The hot topic at the meeting was 'planar patch-clamp', a new technology in which the conventional glass patch-clamp pipette is replaced by a hole in a flat substrate that can be made of various materials including glass, polydimethylsiloxane (PDMS), or silicon. The membrane seal is formed where the cell contacts the substrate rather then at the tip of a conventional pipette. Positioning of the cell over the hole is achieved by various means, including electrophoresis, suction and physical positioning. The presentation of data using the planar patch-clamp technologies began with Jia Xu of Aviva Biosciences (San Diego, CA, USA). He presented whole-cell sodium and potassium currents recorded from neuroblastoma and Chinese hamster ovary (CHO) cells, respectively, as well as currents from rat basophilic leukaemia (RBL-1) cells. The substrate being used

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by Aviva is a modified silicon chip with the edges of the hole smoothed by thermo-oxidation, which greatly increases the success rate for seal formation. Aviva report that most cells form seals within 15 sec and the mean values for seals are ~10 GΩ for RBL-1 cells and 4GΩ for CHO cells.

Chris Mathes of Axon Instruments presented whole-cell potassium currents (Kv1.1) measured in neuroblastoma cells using 2 μM apertures in PDMS. Axon is currently developing a high-throughput planar patch-clamp machine (PatchXpress™) with 16 headstages in parallel, capable of both voltage and current clamp, which they expect to launch at the end of 2002. Jan Behrends of Nanion Technologies (Munich, Germany) presented whole-cell calcium and potassium currents measured from neuroblastoma and CHO cells, respectively. Nanion Technologies uses a borosilicate or quartz microstructured substrate with a varied aperture size depending on the cell type. Alfred Stett from Cytocentrics (Reutlingen, Germany) presented success rates of 97% for cell positioning and 68% for gigaseal formation using a novel substrate that contains a larger hole (~40 μM) for cell positioning surrounding a smaller hole (1–6 μM) used for seal formation and recording. Some spontaneous whole-cell access (16%) and additional whole-cell access using suction was also reported.

IonWorks™ platforms that are currently under development at Molecular Devices Corporation (Sunnyvale, CA) were described by Wilhelm Lachnit. The IonWorks™ APC system is an assay development and safety profiling system that offers flexibility and sensitivity to record whole-cell currents or single channel events from cells, whereas the IonWorks™ HT system is a high-throughput system capable of generating data from 1000 to 3000 patches per day in whole-cell mode. Lachnit presented whole-cell potassium currents (Kv1.5 and HERG) expressed in CHO and human embryonic kidney (HEK) cells generated on these platforms, which will be available later this year.

Overall, the explosion of planar patch-clamp data over the past year does much more than validate proof-of-concept. The success of multiple groups using widely varied substrates suggests that the 'planar patch-clamp' technology is feasible and will probably become commercially available in the near future. However, which substrate works best for which applications is yet to be determined. The next 12–18 months should prove the value of these novel technologies by providing an enormous opportunity for pharmaceutical companies to screen pharmaceutical compound libraries directly at the electrophysiological level and, therefore, effectively exploit ion channel targets.