Drug discovery technology for ion channels

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The *Drug Discovery Technology for Ion Channels* satellite meeting of the Biophysical Society 45th Annual Meeting (Cambridge, MA, USA) sponsored by Millennium Pharmaceuticals brought together both academic and pharmaceutical/biotech investigators with a common interest in ion channels and their potential as targets for drug discovery.

The stage was set with a consideration of the crucial roles that ion channels play in numerous (and perhaps all) cell types and why they are viewed as a favorable class of drug targets. Frances Ashcroft (Oxford University, UK) opened the proceedings with an overview of channelopathies (ion-channel-dysfunction underlying diseases) drawn from her excellent new book[1], including work from her own lab on the role of the K_{ATP} channel in insulin secretion. John Wood (University College London, UK) reviewed the data for voltage-gated sodium channels in peripheral sensory neurons as analgesic drug targets, including evidence from studies of knockout mice.

Ion channels occur as large families of related genes with cell-specific expression patterns, raising their utility as tissue-selective drug targets. Rory Curtis (Millennium Pharmaceuticals, Cambridge, MA, USA) considered genomic methods for mining these gene families for new target discovery, especially in light of the recent publication of the human genome. The utility of transcription profiling using DNA microarrays to understand the tissue distribution of ion channels and their role in physiological and pathological states was also briefly described.

Technologies for ion channel screening

Specific presentations covering ion channel screening technologies included established technologies as well as emerging technologies. The FLIPR® (Fluorometric Imaging Plate Reader; Molecular Devices, Sunnyvale, CA, USA) and VIPR™ (Voltage Ion Plate Reader; Aurora Biosciences, San Diego, CA, USA) were two of the established platforms described. Michael Xie (Millennium Pharmaceuticals) illustrated the successful use of FLIPR® and the Membrane Potential Kit (a fluorescent reagent from Molecular Devices) in developing assays for voltage-gated potassium channels, ligand-gated calcium channels and electrogenic transporters. The Membrane Potential Kit reagent eliminates wash steps and has faster response times than DiBAC4(3), thereby making high-throughput membrane potential assays a reality. Tito Gonzales (Aurora Biosciences) described their ion channel screening platform, which includes their new VIPR™ II, and their voltage sensor probes. The VIPR™ II reader, which is now 384-well-plate compatible and has temperature control, was designed for use with their proprietary FRET-based voltage sensor probe system. This probe monitors membrane potential ratiometrically and is very fast, sensitive and bright.

In contrast to these established platforms, which use fluorescent reporters to measure ion channel activity, Axon Instruments (Union City, CA, USA), a veteran and leader in systems for classical electrophysiological ion channel research, has two new platforms under development which were described by Andy Blatz (Axon Instruments). The first is RoboClamp , which employs a two-microelectrode voltage clamp technique that is useful for directly measuring ion channel currents in oocytes that express the ion channel of interest. The system is currently capable of running eight oocytes simultaneously and is under evaluation with their beta partners. RoboPatch , the second platform described, utilizes a novel electrode fabrication technology developed at Yale University (New Haven, CT, USA). This technology should make possible a 'Patch-on-a-chip' device enabling multiple, simultaneous, single-cell electrical recordings. However, this device still requires a significant amount of development and is probably two years away from commercialization. The MonoPatch presented by Christian Schmidt from Cytion (Epalinges, Switzerland) represented a second 'patch' device, which uses a new cell positioning technique that can measure ion channel currents from membrane vesicles as well as whole cells. This system is expected to be under beta evaluation by the middle of the year. Further iterations of this technology include integration of multiple recording sites on one chip making it suitable for large-scale industrialized screening.

Poster presentations by CeNeS Pharmaceuticals (Cambridge, UK) and Sophion Bioscience (Ballerup, Denmark) displaying their Autopatch™ and Neuropatch™, respectively, demonstrated the development of fully-automated patch clamp systems that do not require trained electrophysiologists to operate. Further development of these systems include parallelization of these platforms, which will increase throughput substantially.

Discovery of ion-channel drugs

Earlier discussions introduced ion channels as important targets for drug development, and outlined new technologies that are in development or are currently being applied to ion channel drug discovery. The final three presentations of the symposium focussed on how integration of these components are being exploited by the biotechnology and pharmaceutical industries to identify and develop ion channel

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drugs successfully. Neil Castle reported that Icagen (Durham, NC, USA) is approaching completion of the cloning of all human ion channel pore-forming subunit genes, which will soon permit the use of focussed ion channel DNA microarrays to examine differences in distribution and expression in healthy and diseased tissue. Furthermore, access to all genes is enabling functional expression of heteromultimeric ion channels, which more faithfully reflect the composition of ion channels found in human cells. The importance of this for ion channel drug discovery was illustrated by showing the different sensitivities to activation of two closely related heteromultimeric voltage-gated potassium channels by two small molecule openers. The identification and functional expression of ion channels from the same gene family, as well as unrelated ion channels, was reported to be invaluable for developing selectivity profiles for drug candidates.

Several speakers illustrated how fluorescence assays using FLIPR® or VIPR™ are being used, or have been been utilized successfully in ion channel drug discovery campaigns. Wilhelm Lachnit (Molecular Devices) presented data on how FLIPR was used at Roche Bioscience (Palo Alto, CA, USA) to develop assays for the identification of small molecule modulators of purinergic P2X ionotropic receptors by measuring changes in intracellular calcium. In a similar vein, Thomas Connolly (Merck, Whitehouse Station, NJ, USA) showed how FLIPR® was used to identify novel and selective antagonists of NR2B glutamate ionophoretic receptors, which were subsequently shown to produce analgesia in several animal models. Tito Gonzalez illustrated how FRET voltage-sensitive dyes were being used in conjunction with VIPR™ to identify novel small-molecule modulators of CFTR chloride channels as a part of a collaboration with the Cystic Fibrosis Foundation (Bethesda, MD, USA). Neil Castle then summarized the successful discovery and ongoing clinical development of an inhibitor of the intermediate-conductance calcium-activated potassium channel (Gardos Channel) in red blood cells for the treatment of Sickle cell anaemia.

Although the majority of the meeting focussed on genomics for discovery and validation of ion channels and the new technologies for HTS, Neil Castle also discussed the importance of chemistry in ion channel drug discovery. He noted that ion channels are quite 'promiscuous' in terms of their ability to be modulated by small, often hydrophobic molecules, which might be related to similarities in the overall structure of the conduction pathway (or 'pore') of ion channels. The challenge is therefore to design chemical libraries with sufficient diversity and 'drug-like' physiochemical properties, which when coupled with the new screening technologies and a complete set of ion channel targets, should improve the identification of quality lead candidates earlier in the drug discovery process.

Reference

1 Ashcroft, F. (2000) *Ion Channels and Disease: Channelopathies*. Academic Press