

The Eighth Annual Ion Channel Retreat, Vancouver, Canada, June 28–30, 2010

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ABSTRACT

Eight years ago Aurora Biomed Inc. (Vancouver, Canada) committed to gathering the brightest minds and the most innovative research companies at one conference. We sought to provide a podium for scientific discourse spanning a wide range of ion channel disciplines. Since then, researchers from both academia and industry have come together each year to share their knowledge. With attendees from 17 different countries at the 2010 Ion Channel Retreat, this conference continues to grow, and is a testimony to the importance of ion channel research. Aurora Biomed's 2010 Retreat covered a variety of topics, including Ion Channels as Disease Targets, Ion Channels as Pain Targets, K-Channels, TRP-channels, Ion Channel Screening Technologies, Ion Channels in Safety Pharmacology, and Structure and Function of Ion Channels.

INTRODUCTION

Over the past year, lingering effects from the global economic downturn have resulted in numerous changes throughout the pharmaceutical and biotechnological industries. Joint collaborations have pooled energy and resources. From the mergers, collaborations, and acquisitions that have taken place, to the subtle streamlining efforts undertaken to reduce costs, avoid risk, and increase the short-term return on investments, effects continue to be felt throughout the field. Mergers and acquisitions enhance long term profitability by streamlining costs and amalgamating key features, thereby providing more comprehensive suite of capabilities. Acquisitions of profiling and drug-assessment services enable the resultant biotechnology company to target specific candidates and support research for better drug candidates.

As mentioned by Dr. John Dunlop (Pfizer), such consolidations are a testimony to the fact that investors still believe in the value of research and discovery and that ion channel research is a vitally important field. To address these issues, Aurora Biomed relied on the guidance of its Scientific Advisory Board to assemble a program that spanned a rich and diverse range of ion channel research topics to be

discussed at the 8th Annual Ion Channel Retreat. The 2010 Scientific Advisory Board was composed of Dr. Alan Wickenden (Johnson & Johnson), Dr. Andreas Jeromin (Banyan Biomarkers), Dr. Gul Erdemli (Novartis), Dr. John Dunlop (Pfizer), and Dr. Peter McNaughton (University of Cambridge). Held in Vancouver, BC, from June 28 to 30, the 8th Annual Retreat welcomed scientists from a variety of research laboratories representing nonprofit and academic institutions and biotechnology and pharmaceutical industries. A total of 127 attendees were present—representing 17 different countries.

The program presented at the 2010 Ion Channel Retreat showcased both the importance of new drug discovery as well as the importance of producing new screening technologies while maintaining or improving safety and efficacy of target compounds. The purpose of this meeting review is to provide a general overview of the conference proceedings. Due to confidentiality requirements for ongoing research projects, the summaries given here may lack a level of substantial detail. Please contact the speakers directly for further information.

ION CHANNELS AS DISEASE TARGETS

Pharmaceutical companies are faced with drugs going off-patent, so new research is needed to fuel the pipeline. This session discussed recent work by researchers attempting to find solutions to address diseases such as schizophrenia, Alzheimer's disease, and cystic fibrosis.

Dr. Dunlop opened the session with a discussion of alpha-7 nicotinic acetylcholine receptors ($\alpha 7$ nAChR), a well-known ligand-gated ion channel. The presence of these channels in the cortex and hippocampus makes them a potential therapeutic target for schizophrenia and Alzheimer's disease, and human and animal studies have strongly implicated $\alpha 7$ nAChR agonists across a range of cognitive processes including memory and learning.¹ The study investigated $\alpha 7$ nAChR agonists and their interaction with positive allosteric modulators (PAM). They used the Dynaflo system (Celectricon) to characterize distinct profiles generating two groups with dramatically different characteristics, classified as either PAM type 1 or PAM type 2. $\alpha 7$ nAChR PAMs have been classified according to their electrophysiological properties: agonist-evoked responses affect the maximum peak current amplitude for type 1 PAMs, while type 2 PAMs alter peak height and receptor desensitization kinetics of the receptor.² Dr. Dunlop used the QPatch system (Sophion Inc.) to show that despite these differences, both $\alpha 7$ nAChR agonists and PAMs had

ABBREVIATIONS: ASICs, acid-sensing ion channels; CBX, carbenoxolone; CFTR, cystic fibrosis transmembrane conductance regulator; EP, electrophysiology; GABA, γ -aminobutyric acid; hERG, human *Ether-à-go-go* related gene; iPSC, induced pluripotent stem cells; IS, immunological synapse; nAChR, nicotinic acetylcholine receptor; PAM, positive allosteric modulators; PSC, pluripotent stem cells; QT₁; SLE, Systemic Lupus Erythematosus; SMCs, smooth muscle cells; TRP, transient receptor protein.

the same pharmacological significance. The most interesting similarity was a profound temperature dependence exhibited by both PAMs and $\alpha 7$ nAChR agonists. From this he concluded that the manner in which each group are activated, despite the dramatic differences in their properties (multiple chemotypes, full and partial agonists, and profound difference in desensitization recovery), is different but that they share the same *in vivo* efficacy profiles. Dr. Dunlop emphasized that it is possible to activate the receptor in many ways and still identify the efficacy of agents in preclinical trials. Ultimately it should be possible to successfully target $\alpha 7$ drug discovery, but we must treat clinical data cautiously as efficacy profiles may be misleading.

Dr. Fredrick Van Goor (Vertex Pharmaceuticals) introduced the cystic fibrosis transmembrane conductance regulator (CFTR), a protein kinase A-activated Cl^- channel. Mutations in the CFTR cause a reduced CFTR-mediated chloride transport and a subsequent increase in the sodium flux. This results in airway degradation and reduced ciliary beating in cystic fibrosis patients. Dr. Van Goor discussed two approaches for therapies that were pursued by Vertex Pharmaceuticals. The first focused on identifying correctors that improved the processing and synthesis of CFTR by providing more compact folding and therefore decreased the likelihood of degradation. The second method was to identify potentiators, which increased channel gating activity and subsequently increased Cl^- transport, thereby restoring the ion channel balance. Both methods increased the ciliary beat frequency in the lungs, which indicated that they were a viable therapy target. Dr. Van Goor indicated that when used in conjunction with each other, these may prove to be very significant therapies for lung-related disease in cystic fibrosis patients.

ION CHANNELS AS PAIN TARGETS

Dr. Alan Wickenden (Johnson & Johnson) introduced this session with two questions: (i) Which is the best molecular pain target? (ii) Which are the most safe and analgesic compounds to target? Each of the talks in this section highlighted the fact that these questions require consideration of both the experimental methodology and the critical cellular mechanisms being regulated. These presentations reflected both the difficulties scientists encountered and the success stories that have come out of recent investigations into the elusive role of ion channels as pain targets.

Dr. Birgit Priest (Merck Research Laboratories) reviewed the role of Na^+ channel blockers as analgesics. In particular, she discussed the notoriously difficult target, Nav1.7, and the critical role that this channel plays in pain signaling. Dr. Priest's research focused on alternative-state-dependent Nav1.7 blockers with hopes of discovering a compound similar to the well-known blocker lidocaine, which has a minimum effective concentration of 16 μM . She presented a subtype selective, state-dependent compound, biphenyl triazoline, which displayed all the indicators for efficacy in preliminary investigations, including a minimum *in vitro* effective concentration of 0.2 μM , but, surprisingly, no sign of efficacy in clinical trials. This posed the question: How does one avoid this result? To answer this question, Priest discussed how to look for red flags in the data. In this case, the triazoline compound is 100-fold more potent than lidocaine in

the absence of protein during *in vitro* testing, but unlike lidocaine, triazoline showed high protein binding, poor pharmacokinetics/pharmacodynamics (PK/PD) correlation, and a therapeutic window that may have been too good to be true. Dr. Priest hypothesized that PK values could be used to estimate efficacy, and should be used in conjunction with threshold tracking to ensure reproducibility. In conclusion, Priest suggested that future clinical trials would require molecules with better properties. She felt that threshold tracking reproducibility was identified as one such path for testing molecules; Priest recommended that in subsequent investigations it may be useful to examine certain properties such as protein binding before proceeding with clinical trials.

Dr. Jianguo Cheng (Cleveland Clinic) continued the discussion along a similar vein. His team studied neuropathic pain and the recent recognition in the literature of the critical role of gap junctions in the maintenance of pain facilitation and the management of neuropathic pain. Cheng discussed the effects of two carbenoxolone (CBX, a widely used gap junction blocker) analogs: 18- α -glycyrrhetic acid (a gap junction blocker) and glycyrrhizic acid (which lacks the gap junction decoupling property). After investigation they found no change in withdrawal latency for either 18- α -glycyrrhetic acid or glycyrrhizic acid, indicating that CBX has unique properties in conjunction with its gap junction decoupling capability. He concluded that the properties of CBX, such as its antioxidant and anti-inflammatory effects, should be investigated, as this may lead to a better understanding of neuropathic mechanisms.

Kambiz Shekdar (Chromocell Corporation) discussed the benefits of taking a well-studied protein, such as Nav1.7, and investigating the individual entities of this multi-subunit ion channel. This method is useful because it opens the door to information regarding the dependence of ion channel pharmacology on subunit variability's. Dr. Shekdar was interested to discover the structure of this Nav1.7 channel *in vivo*, and whether there could potentially be different forms of this target. Dr. Shekdar's research tested millions of clones to isolate rare optimal cells that were positive for each subunit. Cells were initially characterized on the functional level using a membrane potential assay and then subsequently characterized using electrophysiology. It was found that the alpha-only cell lines did not demonstrate the same characterization as the intact multi-subunit cell line. Distinct pharmacology resulted from different clones each expressing the same alpha-beta subunits. As a result of this campaign, Dr. Shekdar presented 20 structurally distinct chemical series and highlighted the informational importance of each individual subunit. By using the standard filtering techniques, while focusing on the underlying biology, his group identified 180 distinct chemical scaffolds. Dr. Shekdar concluded that cross-comparative pharmacological characterization of numerous multi-subunit cell lines may be integral in generating potent and efficacious leads for other complex targets.

K CHANNELS: LINKS TO PAIN AND DISEASE

Dr. Laura Conforti (University of Cincinnati) introduced the role of K^+ channels in pain and disease by highlighting the connection

between Systemic Lupus Erythematosus (SLE), an autoimmune disease, and Kv1.3 channels. She explained that a hallmark of the disease is an exaggerated Ca^{2+} response. This is thought to be controlled primarily by Kv1.3 channels in human T lymphocytes. Thus, many therapeutic treatments have been focused on downstream modulation of this channel in T and B cells. Dr. Conforti presented data showing that there was no difference in the expression levels of Kv1.3 channels between normal T cells and SLE T cells. These channels also had the same pharmacological features. A complex communication existed between T cells and receptor B cells through various cytokines and physical interactions. Dr. Conforti explained that the immunological synapse (IS) contained membrane molecules such as T cell receptors and signaling molecules such as cytokines. The IS facilitated and regulated signaling between the T cell and the antigen presenting cell, and by modulating Kv1.3 activity. In normal individuals the Kv1.3 channel localized into the IS for up to 2 hours. In SLE, a defect in the Kv1.3 channel localization reduced channel localization, which resulted in an exaggerated Ca^{2+} response. Therefore, ion channel distribution in the IS played a critical role in the stabilization of Ca^{2+} influx. Dr. Conforti concluded that the IS controlled the magnitude and duration of Ca^{2+} response and thus ultimately regulated T cell activation.

Dr. Kenneth Byron (Loyola University) continued the discussion on voltage-gated potassium channels by introducing KCNQ (Kv7) channels in smooth muscle cells (SMC) within the walls of the arteries and bronchioles. SMCs control blood pressure and respond to changes in Ca^{2+} concentration. Dr. Byron's group looked at the Ca^{2+} responses mediated through different mechanisms and found that KCNQ currents are an intermediate in this pathway. Dr. Byron explained that they found Kv7 channels contributed to the stabilization of resting membrane potential and signal transduction. From this he showed that KCNQ channel modulation affected blood pressure. Drugs that activated the KCNQ currents induced vasodilatation, whereas drugs that inhibited KCNQ currents induced vasoconstriction. Investigating KCNQ channel interactions may be relevant to future clinical screening trials as certain drugs in clinical use have unexpected results due to previously unrecognized interactions with vascular KCNQ channels. Dr. Byron concluded that SMC Kv7 channels have great potential as future therapeutic drug targets.

Dr. Jerod Denton (Vanderbilt University) closed the session with a discussion concerning the renal outer medullary potassium channel, ROMK (Kir1.1, KCNJ1). ROMK is known to regulate Na^+ , K^+ , and water balance. Mutations in this channel cause diseases, including Bartter syndrome, resulting in excessive urination, low blood pressure, and most importantly, low potassium plasma levels. Interestingly, Dr. Denton discovered that heterozygous carriers of the ROMK mutation had lower blood pressure but no other signs of Bartter's disease, including normal potassium concentrations in blood plasma. Data from another group was also presented, where SNPs in putative KCNJ regulatory regions also resulted in lower blood pressure but not decreased potassium levels.³ Thus, he hypothesized that a partial antagonist of the ROMK could be a novel diuretic. Through a high-throughput screening campaign of approximately 125,000 small

molecules, compound VU590 was revealed to inhibit ROMK at a very slow rate. By examining the selectivity of VU590 Dr. Denton established that it inhibited Kir7.1 as well as ROMK. VU591 was subsequently developed by combining mutagenesis and electrophysiology methods. VU591 was stable, potent, and selective to ROMK over many other off-targets, including the entire Kir channel family. Dr. Denton concluded that this new highly selective probe provides a useful tool for understanding Kir7.1 and the therapeutic benefits of ROMK.

TRANSIENT RECEPTOR PROTEIN CHANNELS: LINKS TO PAIN AND DISEASE

Previous studies have indicated that transient receptor protein (TRP) channels are activated and regulated by a wide variety of stimuli. TRP channels provide a potential target for pain and disease therapeutics. Dr. Sven-Eric Jordt (Yale University of Medicine) initiated the discussion about TRP channels. Dr. Jordt discussed asthma, a disorder caused by overly protective immune responses upon exposure to allergens and irritants, such as the aldehyde acrolein. Most protective responses can be blocked by capsaicin; however, this pretreatment has limited efficacy in many cases because of the occurrence of further downstream mechanisms. TRPV1 is an irritant-sensing, nonselective capsaicin cation channel which promotes inflammatory responses. TRPV1 has been eliminated as an electrophilic target; however, previously published pharmacological data suggest that a homolog of TRPV1, TRPA1, mediates the sensory neuropathic effects of mustard oil. Dr. Jordt presented that TRPA1-deficient mice were not activated by acrolein, which implied that TRPA1 could be a sensor for environmental irritants. They tested mice with a TRPA1 antagonist, HC-030031, and found decreased airway hyperactivity during allergen exposure. Dr. Jordt concluded that TRPA1 is a major irritant receptor in the airways and an important therapeutic target for inflammatory disorders.

Dr. Felix Viana de la Iglesia (Universidad Miguel Hernandez) introduced another member of the TRP family, TRPM8. This nonselective cation channel is expressed in a small population of sensory neurons. TRPM8 plays a critical role as a cold receptor in the detection of mild cold temperatures. The thermal threshold defines the functional phenotype of cold-sensitive fibers, resulting in either pleasant or noxious sensations being evoked. Dr. De la Iglesia initially researched the differences between native TRPM8 and TRPM8 knockout systems and found that the thermal threshold differs between *in vitro* and *in vivo* systems. Native channels were much more susceptible to changes in temperature threshold when cooling and menthol was applied, leading to the suspicion that other modulators were involved. Using site-directed mutagenesis Dr. de la Iglesia demonstrated that the configuration of the channel at the menthol binding site was essential for inhibition but that a mutation at this site did not affect the inhibition of other antagonists. He concluded that there are two different binding sites on the TRPM8 channel that play a role in antagonist inhibition.

ION CHANNEL SCREENING TECHNOLOGIES

In the last year, a whole new generation of technology has emerged. As noted by Alan Wickenden from Johnson & Johnson, ion channel screening technologies are no longer limited to instrumentation, and now include developments in reagents, cell lines, and off-the-shelf assays. The pace of progress has been steady throughout the past year—making this an exciting time. Theoretically, such technological breakthroughs will remove existing impediments preventing us from finding ion channel targeted drugs.

Fittingly, Dr. James Costantin (Molecular Devices) initiated the Ion Channel Screening Technologies session by unveiling his team's newest high-throughput IonWorks instrument. The IonWorks Barracuda, set to launch at the end of the year, is expected to be at the forefront of ion channel screening technologies. This new system enabled the analysis of both ligand-gated channels as well as voltage-gated channels, and resulted in improved data quality and cost reductions. The Barracuda measured cell membrane currents using a patch clamp technique similar to the previous IonWorks Quattro system. However, it also added additional features, including a 6-h walk away time, ~10,000 data points per day, and the capacity for eight compounds per well. Dr. Costantin presented validation data from the instrument for both ligand-gated channels and voltage-gated channels. Validated cell lines included nAChRs, acid-sensing ion channels (ASICs), γ -aminobutyric acid (GABA) chloride channels, as well as NaV and human *Ether-à-go-go* related gene (hERG) channels. Dr. Costantin showed that these cell lines reported consistent results using the IonWorks Barracuda.

Dr. Sikander Gill (Aurora Biomed) linked this session to the previous day's TRP Channel session by presenting data from a new screening assay recently developed using Aurora's Ion Channel Reader 8000 (ICR8000). The TRPC3 receptor serves as a uniquely multifunctional signal transducer, providing cellular Ca^{2+} signaling via multiple mechanisms and may control a variety of distinct physiological functions. TRPC3 may therefore be a potential therapeutic target for selective pharmacological interventions. TRPC3 channels were activated using either 1 μ M of ATP or carbachol. The high-throughput screening assay was optimized for various factors, including ionic composition of buffers, pH, and activation period. Optimal values were found to be pH 7.4 with a 6-min activation period. IC_{50} values for compounds such as Pyr3 (1.44 μ M), flufenamic acid (7.48 μ M), 2-aminoethoxydiphenyl borate (6.09 μ M), gadolinium (0.144 μ M), and lanthanum salt (2.88 μ M) were determined. In conclusion, this assay showed that Ion Channel Reader technology is applicable to TRPC3 channels and indicated that the Li^+ influx assay may be applicable to other members of the TRP family.

Dr. Rodolfo Haedo (Nanion Technologies GmbH) began his discussion by reviewing the current Nanion Port-a-Patch and its higher-throughput sister, the Patchliner. Dr. Haedo explained that both systems allow patch clamp recordings at temperatures ranging between 55°C and 75°C for both ligand- and voltage-gated channels, allowing a wide range of experimental freedom to the user. To confirm this, both systems were validated using several Chinese hamster ovary and human embryonic kidney cell lines as

well as primary cells, such as mitochondria and smooth muscle cells, and stem cells. Pooled data from the systems showed consistently high success rates for both gigaseal and long-term recording comparisons between planar and conventional patch clamp technology. He noted that they achieved excellent results even with cell lines and ion channels notorious for being difficult to work with on conventional units, such as mitochondria and erythrocytes. Data were presented from experimental applications of temperature-controlled patch clamps to heat-activated TRPV1 channels and stem cell-derived cardiomyocytes. These experiments yielded good results in terms of recordings and potency values. In conclusion, Dr. Haedo showed that their planar patch clamp technique provided more experimental flexibility than conventional methods while maintaining consistent IC_{50} values.

Dr. Stephen Hess (Millipore) discussed Millipore's cell-based assay capabilities, their continuous validation of new ion channel relevant cell-lines, and their performance assessment of ready-to-assay frozen cells. Dr. Hess presented validation data and pharmacological characterization of ion channels from Millipore's catalog of stable ion channel cell lines. These included GABA α 5, ASIC3, CFTR, Kv12.2, and Kv7. Validations were performed using both manual patch clamp and various automated patch clamp platforms. Millipore plans to release several newer cell lines in the near future. To conclude, Dr. Hess touched on ready-to-assay frozen cells. Validation results were obtained using an IonWorks assay. Based on the positive results of these assays, Dr. Hess concluded that Millipore is confident the cells would also be suitable for use on other screening technology platforms.

Dr. Rikke Schröder (Sophion Bioscience) introduced the new QPatch HTX system from Sophion. Recently, Sophion added a multi-hole patch technology to their QPatch product line. Dr. Schröder presented that this novel technology enabled true gigaseal recordings of up to 10 cells patch-clamped in parallel. Dr. Schröder specified that this technology is useful for assays with large cell-to-cell heterogeneity as well as transiently transfected cell lines. Dr. Schröder presented the biophysical and pharmacological characterization of four very different ligand-gated ion channels. Her research group looked at single- and multi-hole data for rise time, desensitization, and pharmacology for the nAChR α 1 (TE671 nAChR α 1), ASIC 1a (ASIC1a), glutamate receptor 5, and GABA receptor A (GABA $_A$). Results were consistent with high success rates. This was especially clear with GABA $_A$ for which the multi-hole method increased the success rate from 37% to 93% in comparison to single-hole data. Dr. Schröder concluded that multi-hole patch technology is a valid and advantageous method for ligand- and voltage-gated channels.

Dr. Clemens Möller (Evotec AG) had previously presented data on their investigations of *in silico* two-dimensional (2D) and three-dimensional (3D) pharmacophore models for predicting hERG liabilities at the 2009 Ion Channel Retreat. At that time, he discussed difficulties they had faced and what modifications would be required to produce a successful model. They had concluded that 2D *in silico* models were not successful. At this year's Ion Channel Retreat, Dr. Andreas Ebnet (Evotec AG) discussed a modified approach:

hERG 3D *in silico* modeling. Dr. Ebnet demonstrated that this modeling method is more effective than 2D *in silico* modeling and correctly identified hERG liabilities, allowing for their subsequent removal using minor structural changes. In their investigation of the histamine H3 receptor (notorious for hERG liabilities), this model was highly predictive. This novel modeling approach assisted in their discovery of a compound that was highly potent to the H3 receptor and devoid of hERG inhibition. This H3 receptor will be moving forward as a clinical investigation candidate. He concluded by noting that Evotec was close to publishing documentation regarding this hERG *in silico* 3D modeling technique.

Dr. Andrew Southan (Biofocus) continued the discussion regarding recent areas of rapid advancement by presenting their validation of Celectricon's Dynaflo[®] HT system. Dr. Michael Dabrowski (AstraZeneca) had presented this system in its final stages of development during the 2009 Ion Channel Retreat. At this year's conference, Dr. Southan discussed Biofocus' laboratory experience with this novel technology. Although EP-based screening has been widely accepted for voltage-gated channel targets, ligand-gated targets remained challenging. Dr. Southan presented results from testing GABA_A $\alpha_x\beta_Y\gamma_Z$ and Kv1.5 using the Dynaflo HT system. In this study, five plates were examined over several days for mean current amplitudes and pharmacology. Dr. Southan illustrated that even without optimization, when comparing data to historical results, they achieved great results and consistent performance from unattended runs. He concluded that Celectricon's Dynaflo HT system was a promising solution for ligand-gated channel targets.

Dr. Ralf Kettenhofen (Axiogenesis AG) introduced pluripotent stem cells (PSC) and the properties that make them beneficial for advanced electrophysiological drug screening. PSCs have the advantages of recombinant cell lines (ease of use, high-throughput capability, and standardization abilities) while lacking the downfalls associated with primary cardiac myocytes. Dr. Kettenhofen discussed two specific ways in which PSCs are more advantageous. The first is self-renewal and indefinite growth. The second is the possibility of specification of a pure population. Dr. Kettenhofen's data investigated both large scale productions of mouse embryonic stem cells and small-scale productions of human induced PSC (iPSC), which he showed to be comparable systems. By comparing the pharmacological properties of these PSCs to data from known compounds active on cardiac cells, Kettenhofen provided evidence that mouse embryonic stem cells as well as human iPSC-derived cardiomyocytes are excellent *in vitro* models for the detection of cardiac ion channel modulation.

Numerous speakers presented work on the GABA_A receptor at this year's retreat. Dr. Juha Kammonen (Pfizer Global Research & Development) gave a detailed overview of effective ways to investigate this receptor. Data were presented from experiments using several commercially available assay screening technologies. Dr. Kammonen wished to develop an identification assay for locating new PAM of the GABA_A receptor similar to known therapeutic agents, such as benzodiazepines. His group tested GABA_A $\alpha 1\beta 3\gamma 2$ and $\alpha 2\beta 2\gamma 2$ cell

lines using a planar physiology assay with a QPatch instrument (Sophion Bioscience), current screening using an in-house 96-well filter binding assay, a population patch-clamp assay with an IonWorks Quattro system (Molecular Devices), a FLIPR (Molecular Devices) membrane potential assay, and an automated patch clamp assay using the IonFlux 16 system (Fluxion Biosciences). Dr. Kammonen found that throughput from the QPatch was too low to support structure activity response generation and had low success rates for their assay. The 96-well filter binding assay measured displacement. Throughput and data quality were satisfactory, but data were less physiologically relevant than that provided by functional assays and could not suitably predict efficacy. Dr. Kammonen showed that both the IonWorks Quattro and FLIPR membrane potential assays were inconsistent for their assay. In conclusion, he presented the tool they will be moving ahead with. His research showed the IonFlux 16 provided the highest throughput while maintaining data quality, making it an excellent tool for the investigation of the GABA_A $\alpha 1$ and $\alpha 2$ receptors as well as other ligand- and voltage-gated channels.

Rounding out the ion channel screening technologies session was a talk by Dr. Marzia Martina (National Research Council of Canada). She explained that current chip-based technologies have drawbacks—conventional patch-clamp techniques are labour intensive, require highly trained personnel, and have low throughput. The main deficiencies of current planar patch-clamp techniques include lower quality recordings and limited relevance to physiological investigations. Dr. Martina presented results from her development of a novel technology that would allow researchers to interrogate the electrophysiological activity of individual cells at multiple sites, within cellular networks, with resolution at the ion channel level. She presented high-fidelity patch-clamp recordings of synaptic activity obtained from the first neurons to have ever been cultured directly on silicon patch-clamp chips. Dr. Martina concluded that the silicon planar patch-clamp chip was a promising tool that could allow researchers to obtain high fidelity measurement of synaptic events. This advanced platform provided high-information content interrogation of physiologically and pathophysiologically relevant *in vitro* models.

CARDIAC ION CHANNEL PHARMACOLOGY

As researchers try to understand and explain the many parts of cardiac channels, various aspects have remained elusive despite the large amount of publicity over the past few years. Researchers now strive for more effective screening methods to narrow the pipeline earlier in the process. Investigations are now studying the actual mechanism of hERG channel activity. Presentations in this section evidenced that research has diverged towards new, less conventional directions such as the investigation of long QT syndrome as an important tool for developing drug targets for cardiac arrhythmia.

Dr. Gul Erdemli (Novartis) opened the session by discussing the ongoing efforts to eliminate hERG channel liabilities in drug discovery efforts. She identified the effectiveness of the many methods, primarily *in vitro* assays and *in silico* modeling, which

are used to identify the cardiac safety liabilities. Specifically, Dr. Erdemli discussed the benefits of implementing preclinical *in vitro* ion channel safety profiling in the integrated risk assessment for cardiac safety case studies. Of interest were the hERG channel as well as off-target cardiac ion channel activity such as Nav1.5, Cav1.2, and KCNQ1/minK. Her research covered a clinical proof-of-concept study (increasing doses resulted in PR interval prolongation at 640 mg), which was terminated due to the risk of cardiac conduction abnormalities. Overall, she concluded that the data from this study correlated well with the *in vivo* data as they displayed the translational value of the *in vitro* data, which supported the need for early screening. Dr. Erdemli suggested that one should keep in mind that testing drug effects do not mean everything in regards to drug safety. Future studies should consider properties such as the in-depth modulation of ion channels and other mechanisms (such as trafficking, maturation, degradation, electrolyte levels, and homeostasis).

Complimenting Dr. Erdemli's discussion on techniques for identifying cardiac liabilities, Dr. David Fedida (University of British Columbia) directed the discussion toward the mechanisms of hERG channel activity. He pointed out that hERG activation is surprisingly slow compared with other Kv channels. It is unknown whether slow hERG activation is caused by slow movement of the S4 transmembrane domain or slow coupling of the gating mechanism to pore opening. Dr. Fedida explained that there are three ways to investigate the activation mechanism: Nuclear magnetic resonance, gating currents, and fluorescence spectroscopy. His research showed that both fluorescence at E519C and gating currents from mammalian cells had comparable results. Dr. Fedida presented the first ever recorded mammalian cell results of this nature. Results showed a rapid S4 translocation. This implied that the slow pore opening of hERG channels likely reflected downstream events after S4 movements, which should yield interesting research possibilities in the future.

Dr. Dick Wu (Washington University) moved away from the topic of hERG mechanisms and instead presented a possible therapeutic target for cardiac diseases such as atrial fibrillation and long QT Syndrome. Dr. Wu and his team studied IKs channels, which consist of Kv7.1 and KCNE1 subunits. Mutations in this channel can cause prolonged QT syndrome. Dr. Wu's study focused on the long QT mutation E1K, which inhibits channel function. Conserved charged glutamate (E) residues in the S2 and S4 segments of the Kv7.1 voltage sensing domain (VSD) are thought to interact with conserved arginine (R) residues, thereby potentially stabilizing channel structure or assisting in channel activation. The E1 mutation to a glycine residue results in a charge reversal within the VSD. He showed that KCNE1 changed voltage-dependent activation of Kv7.1; the presence of KCNE1 dramatically slowed channel activation. Therefore, the E1K mutation disrupted electrostatic interactions crucial to channel function. Interactions between E1 and R3/R4 resulted in voltage sensor activation, suggesting R4 may interact with E1 at the activated state. Dr. Wu concluded that E1 interacts specifically with the S4

segment and that modulation of this interaction would be a potentially important drug target since KCNE1 was shown to be modified extracellularly by exposing it to different electrostatic environments. He also demonstrated that the IKs channel required KCNE1 to be present for modification; thus, this target offers a high level of specificity while minimizing potential side effects.

Dr. Blake Anson (Cellular Dynamics) wrapped up the session by introducing genetically engineered stem cells and the use of iPSC technology to meet the requirements for large quantities of high-purity human stem cells needed for drug discovery and toxicity testing assays. His group focused on three factors: quantity, quality, and purity. Cellular Dynamics has used this technology to generate cardiomyocyte samples with >99% purity. The purity was initially quantified using reverse transcription polymerase chain reaction. Fluorescence subsequently showed that all appropriate elements were present. Finally, electrophysiological characterization confirmed cardiac action potential and provided an effective method for assessing cardiac ion channel function. To verify utility in drug discovery and toxicity testing, cells were exposed to cardioactive agonists, antagonists, and toxicants. Subsequent recordings provided solid evidence of the significance of iPSCs in industrialized cell generation. Dr. Anson concluded by noting their intention to further investigate neural cells in future studies.

STRUCTURE AND FUNCTION OF ION CHANNELS

Research presented over the duration of the previous six sessions demonstrated that many different important ion channel families exist and that structural changes may alter functionality at specific target sites. The final discussions for the retreat revolved around the properties of various channel isoforms and their properties that could potentially be targeted for further testing.

Dr. James Prudent's (Centrose) presentation centered on the difficulty of finding drugs that target channels without leading to numerous outcomes and potential side effects, due to the multiple mechanisms and activities that depend on any given ion channel's isoform and surrounding subunits. His research offered the novel idea that this problem results from different drug-isoform interactions and that these differences may result in highly specific drugs. Dr. Prudent presented data from studies targeting the Na⁺,K⁺-ATPase pump, which had recently been noted in the literature as a potential target for cancer and inflammation treatments, as well as a therapeutic target for cardiac failure. Dr. Prudent showed that when molecular drug candidates specific to the Na⁺,K⁺-ATPase pump were enhanced with novel carbohydrates, it was clear that trafficking was isoform dependent and that isoform expression was cell selective. Certain molecules may undesirably inhibit trafficking by blocking the binding site on the target compound, causing the drug to dissociate prematurely from the channel, or by preventing binding to the target tissue. From this result, he concluded that by researching targeting trafficking vehicles, such as antibodies, specific to each isoform, scientists might discover a vehicle for which binding to the trafficking molecule does not interfere with drug binding to the ion channel or target. Isoform specific trafficking molecules would

preferentially carry desired compounds through specific channels, thus allowing both drug and vehicle to reach their targets without dissociating. Specifically, Dr. Prudent's research showed that drugs attached to ion pump subunit-specific antibodies did not require internalization, were stable, were capable of targeting drugs specifically to the diseased cells, and, most excitingly, could target ion channels and metastasizing cancer cells.

Dr. Zhiyuan Li (Guangzhou Institute of Biomedicine and Health) closed the session with a final discussion about iPSCs. In his talk, he suggested that iPSCs could mimic the ion channel properties of motor neurons. He explained that the first task was to identify a mechanism of differentiation that enhanced the biology of these cells. After establishing a protocol for neural induction of iPSCs, the iPSC-derived neurons were then identified using immunochemical analysis (antibody staining) and patch clamp recordings. By looking at resultant current clamp recordings for voltage-gated sodium, potassium, and calcium currents, Dr. Li concluded that iPSCs can efficiently generate electrically active motor neurons with the same properties as primary cells.

CONCLUSION

The 2010 Ion Channel Retreat allowed speakers from academia and industry to present various novel approaches to targeting ion channels for potential therapeutic solutions. This year, several talks repeatedly focused on specific ion channel classes—different researchers discussed the TRP channel family, K⁺ channels, and GABA_A receptors throughout the conference. Novel technological platforms such as the silicon planar patch clamp and higher-throughput models of previously available screening platforms have or will soon be launched commercially. These systems provide reliable automated solutions with excellent data quality. Several new stem cell platforms were also introduced that will provide researchers with a renewable and maintainable resource for downstream drug discovery and toxicity testing. New screening technologies and resources continue to revolutionize the marketplace and promise to decrease bottlenecks while increasing output. As drugs go off-patent and pharmaceutical companies strive to find novel therapeutic solutions, automation will continue to lower costs and improve efficacy and safety.

Aurora Biomed's Ion Channel Retreat is a yearly conference that began in 2003 and has consistently provided an environment for researchers and industry leaders to exchange ideas, discuss future collaborations, and consider future research proposals. We thank all the sponsors and attendees for their continued support—this conference has steadily grown each year—and we would like to invite submissions for speaking and poster presentations at the 9th Annual Ion Channel Retreat to be held in the summer of 2011 (dates to be announced).

DISCLOSURE STATEMENT

No competing financial interests exist.

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