The Tenth Annual Ion Channel Retreat, Vancouver, Canada, June 25–27, 2012

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ABSTRACT

Ten years after Aurora Biomed (Vancouver, British Columbia, Canada) hosted the inaugural Ion Channel Retreat, this event is recognized as a leading conference for ion channel researchers. Held annually in Vancouver, this meeting consistently provides an outlet for researchers to share their findings while learning about new concepts, methods, and technologies. Researchers use this forum to discuss and debate a spectrum of topics from ion channel research and technology to drug discovery and safety. The Retreat covered key subjects in the ion channel industry, including ion channels as disease targets, transient receptor protein channels as pain and disease targets, ion channels as pain targets, ion channel structure and function, ion channel screening technologies, cardiac safety and toxicology, and cardiac function and pharmacology.

INTRODUCTION

The past year has been one of great hesitance across most scientific disciplines in both academia and industry. As smaller companies were swallowed up in acquisitions and larger companies were hesitant to invest in R&D, it was uncertain whether this would be reflected in the presentations at the 10th Ion Channel Retreat. Even with these financial constraints, a wide range of innovative research was presented, thus reinforcing the importance of ion channels in drug discovery advancements.

Aurora Biomed appointed the Scientific Advisory Board to use their knowledge and know-how, affiliations, and acumen to create a rich and diverse program of internationally recognized presenters. The 2012 Scientific Advisory Board consisted of Arthur “Buzz” Brown (Chantest), Michael Dabrowski (AstraZeneca), Jerod Denton (Vanderbilt University), Tina Garyantes (MaxSAR Biopharma), Birgit Priest (Eli Lilly), Lu Qiang (WuXi AppTec), and Li Zhang (National Institutes of Health). The Scientific Advisory Board was essential in creating a wide-reaching program, which showcased the importance

of drug discovery research and technological innovations while also exploring ion channel structure and function.

THE TENTH ANNUAL ION CHANNEL RETREAT Ion Channels as Disease Targets

Ion channels represent major targets for a wide range of pathologies due to the vital role they play in many key physiological events. With a large number of drugs going off-patent and a greater understanding of disease pathologies, research continues to focus heavily on ion channels as disease targets.

Dr. Jerod Denton (Assistant Professor of Vanderbilt University School of Medicine) opened the session with studies on renal potassium channels. Two members of the inward rectifier potassium (Kir) channel family, Kir1.1 and Kir4.1, are targets of heritable disease mutations that cause the salt and water-wasting kidney diseases antenatal Bartter and SeSAME/EAST syndromes. In search for small-molecule probes and ultimately novel diuretics, a screen of approximately 225,000 small molecules led to the discovery of three novel Kir1.1 inhibitors: VU590, BNBI, and VU591. In particular, VU591 seems to be a selective and potent inhibitor of Kir1.1, and further *in vivo* studies are currently being conducted. As for novel inhibitors of Kir4.1, a thallium flux assay was developed to screen 3,655 compounds in a pilot screen, which yielded 16 confirmed hits. The team is now preparing for a 300,000-compound screen. In the final portion of his talk, Dr. Denton reported on his search for novel malaria treatments. During blood feeding, mosquitoes double in size and must expel excess water and salts to ensure they can still take off after feeding. As a result, his team's approach is to target urine production. They have identified a small molecule VUXXX, which blocks urine production and causes death in mosquitoes injected with a high potassium buffer intended to mimic the concentration released on digestion of red blood cells. Further screening is being performed to find a selective inhibitor.

Dr. Fumihito Ono (Chief, Section on Model Synaptic Systems, National Institutes of Health) discussed genetic mutations of muscle acetylcholine receptors (AChR), which can cause premature fetal death. His group identified a mutant zebrafish carrying a mutation in the AChR δ subunit as a model for fetal akinesia. This mutation hampers the subunit assembly of the pentameric AChR and prevents the expression of the channel at the plasma membrane, causing

ABBREVIATIONS: APC, anesthetic preconditioning; CaMKII, calmodulin dependent protein kinase II; CBD, cannabidiol; EP, electrophysiology; FSGS, focal segmental glomerulosclerosis; GABA, [gamma]-aminobutyric acid; hERG, human *Ether-à-go-go*-related gene; IR, ischemic-reperfusion; MICE, multiple ion channel effects; MS, multiple sclerosis; nAChR, nicotinic acetylcholine receptor; PAM, positive allosteric modulators; RTX, resiniferatoxin; TdP, *torsade de pointes*; TRP, transient receptor protein; α KAP, alpha kinase anchoring protein.

paralysis in the mutant zebrafish throughout its development. By introducing a wild-type δ subunit gene into the mutant zebrafish, Dr. Ono and his team observed chimeric AChR expression in all muscle cells, which led to a full, functional rescue of the mutant zebrafish, enabling the fish to swim and even produce offspring. However, when the timing of the wild-type δ subunit gene expression was delayed, only partial rescue of the paralyzed fish was observed despite the formation of anatomically normal synapses. Dr. Ono pointed out that the results show promise for gene therapy for the treatment of myasthenic diseases, but the timing of gene expression is crucial for the full rescue.

Dr. Shawn Iadonato (EVP & Chief Scientific Officer, KINETA, Inc.) opened the first of the talks on autoimmune disease treatment. His group is focusing on a specific peptide inhibitor of the Kv1.3 channel, ShK-186, for the treatment of multiple sclerosis (MS). The inhibition of Kv1.3 by ShK-186 abolishes activation and expansion of effector memory T cells and effectively reduces symptoms of disease in animal models of MS. Dr. Iadonato reported the results of the recently completed IND-enabling nonclinical program, which used rodent and primate models as well as human T cells. The program showcased a durable pharmacological response in animals treated with ShK-186, with prolonged efficacy at the injection site. Effective dosage of the drug was also tested. No organ toxicology has been identified for this drug in preclinical toxicological studies in rodents and primates. The team planned to perform phase I clinical trials during the summer of 2012.

Next, Dr. Xin-Ming Shen (Assistant Professor of Neurology, Mayo Clinic) returned to research on nicotinic muscle AChR. He discussed a structure-function relation revealed through two naturally occurring mutations in the α subunit (CHRNA1), G74C and V188M, identified in a patient with congenital myasthenic syndrome. It was found that G74C significantly reduces AChR expression, whereas V188M is robustly expressed in HEK cells. Kinetic studies using patch clamp analysis of single channels revealed that V188M diminishes the apparent channel opening rate and gating efficiency. Since V188 is located at the C-loop, Dr. Shen speculated that the mutation hinders the closure of this loop, which, in turn, hinders the channel gating. This was tested through mutant cycle analysis in which the interactive free energy changes of neighboring residues were calculated in order to determine whether the residues are energetically linked. The study revealed that conserved residues Y190 in the C-loop and D200 in β -strand 10, which connects to the M1 transmembrane domain, are functionally linked to V188, and the mutation V188M impairs the inter-residue coupling of K145 in β -strand 7 with both Y190 and D200. Furthermore, the energetic couplings of V188 with both Y190 and D200 are dependent on K145, thus forming an interdependent tetrad. This observation is consistent with the crystal structure of the mouse AChR α -subunit and indicates that V188M impairs the rearrangement process of the C-loop which initially couples agonist binding to channel gating via the interdependent tetrad.

Furthering the discussion into autoimmune disorders, Dr. Stefan Bittner (Postdoctoral fellow, University Hospital Muenster) revealed his study on the physiology of T lymphocytes and related pathologies,

such as MS. He is particularly interested in TASK channels, K_{2P} channels expressed in T-cell lymphocytes. His model is composed of myelin oligodendrocyte glycoprotein (MOG peptide) inducing an experimental autoimmune encephalomyelitis. Through this animal model of MS, Dr. Bittner showed that either deletion of TASK channels or their inhibition through selective inhibitors improves the course of the disease. In an ongoing study, Dr. Bittner is investigating interaction partners of TASK channels, as well as developing selective inhibitors of these channels as a novel treatment strategy for autoimmune disorders. He speculates that K_{2P} channels are involved in other autoimmune diseases, including rheumatoid arthritis, Crohn's disease, and type I diabetes.

Transient Receptor Protein Channels as Pain and Disease Targets

Originally found in *Drosophila* photoreceptors, the transient receptor protein (TRP) channel superfamily includes a multitude of members. Many of them are involved in sensory and signaling pathways. The TRP channel superfamily represents essential players of many physiological properties, and TRP channels continue to emerge as pharmacological targets.

Dr. Jeffrey Herz (President and CEO, Algomedix, Inc.) opened the session with a discussion on allosteric modulators of TRPA1 channels. The activation of TRPA1 channels in nociceptors leads to both acute neuroinflammatory and chronic pain. TRPA1 is a binding target of various endogenous and exogenous ligands, which share a unique activation mechanism through covalent modifications of Cys residues at the orthosteric site. The goal of Dr. Herz's research is to design novel allosteric antagonists of TRPA1 to target pain at its source in the peripheral nervous system. Through investigations of both endogenous and exogenous mediators, including cyclopentanone prostaglandins, cinnamaldehyde, 4-HNE, A-967079, and fenamate NSAIDs, Dr. Herz highlighted their progress in understanding the mechanism of the action of these regulatory compounds that act at either the orthosteric or allosteric sites. The proposed mechanism for compounds that act on the orthosteric site involves the modification of critical Cys residues and the conformational change in S6 domain near the P loop. By targeting the development of reversible, allosteric TRPA1 compounds, Dr. Herz emphasized that high selectivity and efficacy can be achieved for novel antagonists with therapeutic potential.

Dr. Stuart Dryer (John and Rebecca Moores Distinguished Professor, University of Houston) continued the session by discussing the involvement of TRPC6 channels in nephritic syndromes. Gain-of-function mutations identified in TRPC6 channels lead to the late onset of focal segmental glomerulosclerosis (FSGS) through Ca^{2+} overload in podocytes of the renal glomerulus. Dr. Dryer reported that the gating of podocyte TRPC6 is mechanosensitive, being activated by a hyperosmotic stretch of the membranes in nephritic syndromes. This stretch activation of TRPC6 was observed both in HEK cells and in primary rat podocytes. Knockdown of podocin, a hairpin-loop membrane protein located in the podocyte and whose deficiency mutations also cause FSGS, increased the stretch response

of TRPC6 10 fold. In comparison, in the presence of podocin, TRPC6 is primarily chemosensitive. Having established the critical relationship between TRPC6 and podocin, Dr. Dryer suggested that the role of podocin is also essential in the treatment of glomerular kidney diseases. In addition, he advised the importance of both chemical and mechanical gating of TRPC6 when screening for inhibitors.

Next, Dr. Mike Iadarola (Principal Investigator, National Institutes of Health), revisited TRP channels as targets of pain. The TRPV1 channel, or capsaicin receptor, is responsible for sensing noxious heat and plays a key role in inflammation and depolarizing the primary sensory nociceptive nerve endings. In previous animal studies, Dr. Iadarola has shown that the TRPV1 agonist resiniferatoxin (RTX) possesses therapeutic abilities. An injection of RTX selectively kills cells containing TRPV1, therefore causing the lesion of neurons, nerve fibers, or nerve endings depending on the site of injection, while leaving other sensations, such as touch or mechanical pain, unaffected. Trials of RTX in dogs with bone cancer showed long-term pain control with improved quality of life. At the time of the meeting, Dr. Iadarola had initiated human phase I clinical trials of RTX for targeting cancer pain. He suggested that besides central administration, peripheral RTX administration will be effective in other causes of pain, such as arthritis. Peripheral administration of RTX exhibits localized analgesic actions, which Dr. Iadarola hopes would provide a new direction in personalized pain medicine. Dr. Iadarola is currently investigating positive allosteric modulators (PAMs) of TRPV1, including MRS1477, in preclinical small and large animal models. He has performed a high-throughput screen of more than 300,000 compounds for TRPV1 agonists and allosteric modulators, which has revealed several more chemically distinct TRPV1 PAMs, and these, too, are undergoing medicinal chemical optimization and preclinical evaluation.

Ion Channels as Pain Targets

As seen from the previous session, ion channels play a major role in the pain pathways. The relationship between cannabinoids and pain was accidentally discovered through research on glycine receptors.

The sole speaker for the session, Dr. Li Zhang (Medical Officer, National Institutes of Health), discussed his study of glycine receptors (GlyRs). Using rodent models, Dr. Zhang demonstrated that a major nonpsychoactive component of marijuana, cannabidiol (CBD), significantly suppresses chronic inflammatory and neuropathic pain. The direct interaction between CBD and $\alpha 3$ GlyR at S296 of the third transmembrane domain was established through NMR analysis. *In vivo* studies using $\alpha 3$ GlyR knockout mice proved that the receptor was needed for cannabinoid-induced analgesic effects. Dr. Zhang concluded that $\alpha 3$ GlyRs are a key target for glycinergic cannabinoid-induced suppression of chronic pain that will enable the separation of pain treatment from the side effects of cannabinoids.

Ion Channel Structure and Function

Knowledge of ion channel structures is crucial in understanding their functions, regulatory mechanisms, and modulatory binding

partners. In this session, the focus returned to Kir channels, specifically Kir6.2.

The concluding talk of the day was given by Dr. Harley Kurata (Assistant Professor, University of British Columbia) on ATP-sensitive potassium channels. K_{ATP} channels, or Kir6.2 channels, are unique among all potassium channels in that they are regulated by the ATP:ADP ratio. These channels are the metabolic sensors for insulin secretion and play an essential role in glucose-stimulated insulin secretion. Loss-of-function mutations in K_{ATP} cause hypersecretion of insulin, whereas gain-of-function ATP-insensitive mutations lead to neonatal diabetes. Dr. Kurata's group has investigated the mechanistic link between ligand binding and channel gating in K_{ATP} channels by focusing on the ligand transduction interface, composed of a conserved "slide helix" that forms a junction between the ligand-binding domain in the cytoplasm and the transmembrane pore. Through an alanine scan of residues in this key interface and by monitoring functional effects by rubidium efflux assays, several positions were identified with effects on ATP sensitivity of the channel. The slide helix interface was also found to be extremely sensitive to mutagenesis, with many mutants exhibiting complete loss of function. Dr. Kurata also described an approach to rescue the function of electrically silent mutants, and used this method to demonstrate a critical role for Kir6.2 residue D58, which is conserved throughout Kir channels and acts as an essential coupling element between the slide helix and ligand-binding domain. Mutations of D58 yielded an enormous loss of ATP sensitivity, in which the K_{ATP} channel became virtually insensitive to ATP. Dr. Kurata concluded that this finding serves as a model of how ligand binding is translated to channel gating in Kir channels.

Ion Channel Screening Technologies

Screening technologies are crucial for ion channel studies due to the extensive testing of modulatory molecules and their targets. This was the meeting's longest session that reinforced the importance of ion channel screening technology to research. There is consistently a high demand for the improvement of these methods. During this section, a variety of new approaches for high-throughput and cost-effective screening technologies were discussed. In particular, the challenge of achieving high-throughput, fast-solution exchange to study ligand-gated ion channels was addressed.

Dr. Jeffrey Zidichouski (Senior Research Officer, National Research Council Canada) opened the session by presenting his novel *ex vivo* focal ischemic stroke model. Focal ischemic stroke accounts for 80% of strokes that present in the clinic and is the third leading cause of death in Canada. Despite the demand for improved treatments, the most accurate study models of the condition are limited to *in vivo* animal-based stroke models, which are technically and financially demanding, and *in vitro* cell-culture-based models, which are of low cost but poorly mimic the phenomenon. In answer for the need of more physiologically relevant focal ischemic stroke models for drug discovery, Dr. Zidichouski and his research collaborators have developed an *ex vivo* rat cortical brain slice-based approach that can serve as a more physiological relevant model for the initiation and

progression of focal ischemia as compared with using cell-culture-based models. The new model has been validated through the use of the whole cell patch clamp electrophysiological technique in real time, as a brain slice is subjected to a focal oxygen/glucose deprivation insult. They also showed that pharmacological intervention slowed the rate of progression (depolarization in single neurons recorded within the focal area) and reduced the total stroke volume as subsequently assessed by tissue fixing and TTC staining. The validation showed that the new model can mimic the time-dependent development of focal ischemia that is observed using *in vivo* models. Dr. Zidichouski concluded that the new model serves as a more physiologically relevant (as compared with cell-culture-based models) and more cost-effective technique (as compared with *in vivo* models) for drug screening and validation while also providing a new approach to study how damage occurs and spreads outward from the core ischemic region to adversely affect healthy tissue during a typical focal ischemic event.

In the next talk, Mr. Chris Benjamin (Team Lead Ion Channel Service Operations, EMD Millipore) took to a more focused method of studying ligand-gated ion channels. Although ligand-gated ion channels represent a vast therapeutic target class, current screening methods are better suited to studying voltage-gated ion channels. In order to facilitate research in ligand-gated ion channels, EMD-Millipore has developed cell lines co-expressing all six GABA_A α -subunits individually with the β 3 and γ 2 subunits. He reported on the validation of two of the new cell lines, GABA_A α 1 β 3 γ 2 and α 6 β 3 γ 2. GABA_A profiling assays were performed across multiple platforms for comparison, including manual patch clamp, MDC PatchXpress Assays, MDC IonWorks Quattro, and the latest addition, the MDC IonWorks Barracuda. He concluded that the new Barracuda platform is an appropriate platform for assessing GABA_A functional activity. He plans to extend the GABA_A validation studies to the other four cell lines.

Dr. Elaine Gay (Research Pharmacologist, RTI International, speaking on behalf of Fluxion Bioscience) continued the discussion on the development and optimization of automated electrophysiological assays for ligand-gated ion channels using neuronal nicotinic acetylcholine receptors (nAChR). Dr. Gay performed nAChR response characterizations using α 4 β 2 nAChR expressing SH-EP1 cells on the IonFlux16 platform. EC₅₀ and IC₅₀ data obtained by the automated platform were consistent with conventional electrophysiological assays. The automated recordings using IonFlux16 yielded a high success rate of 93%. The data showed that IonFlux16 is suitable for high-throughput screening of α 4 β 2 nAChR ligands. Dr. Gay concluded that the optimized automated electrophysiological assay for nAChR would enhance the rapid discovery and development of novel ligands and therapeutics.

Next, Dr. Laszlo Kiss (Director, US Discovery, Essen BioScience) reintroduced the latest generation of the IonWorks platform, the Barracuda. Dr. Kiss emphasized the capability of the Barracuda to run automated, complex protocols that allow for a high-throughput analysis of the functional pharmacology of both voltage- and ligand-gated ion channels. Example data on voltage-gated ion channels were shown on assays using voltage-gated sodium channels (Na_v) 1.7

and 1.8. The stability produced by the recordings on the Barracuda was demonstrated using activation and inactivation properties of these channels. Several protocols for measuring the diverse mechanisms of inhibition were shown, including a full inactivation pulse protocol, a partial inactivation protocol, and a pulse train protocol for tetracaine pharmacology. Dr. Kiss then demonstrated example data on ligand-gated ion channels using [gamma]-aminobutyric acid (GABA) receptors. GABA assays revealed agonist and antagonist pharmacology and PAM pharmacology, with comparable results to Dr. Benjamin's data. From his research, Dr. Kiss concluded that the Barracuda platform enables high-throughput mechanistic pharmacology studies.

Advances in automated electrophysiology (EP) technologies, regardless of the instruments and types of biological details, often generate overwhelming amounts of data. In the next talk, Dr. Stephan Steigle (Scientific Account Manager, Genedata AG) discussed how the current data analysis workflow is a limiting factor in the automated patch clamp progress. To satisfy the need for fast and precise data analysis in high-throughput assays, two distinct workflows have been implemented in a screening analysis platform, the Genedata Screener. One workflow covers "single-compound" analysis, while the other examines "multi-compound" measurements. These workflows were demonstrated using a GABA_A modulator assay for the single-compound analysis and a Na_v1.8 inhibition assay for the "multi-compound" analysis. Dr. Steigle concluded that this new workflow provides improved data analysis for today's most complex automated EP experiments, whereby analysis quality is improved while analysis time is decreased. His team is currently working on kinetic fit models to further improve this workflow.

In the following talk, Mr. Rodolfo Haedo (General Manager, Nanion Technologies) reported on the development of a ligand-gated automated patch clamp assay developed on the Patchliner, specifically, neuronal nAChR. Despite the attraction of nAChR as potential ion channel targets for various neuronal diseases, challenges still remain in studying nAChR activity in high throughput due to ultra-fast desensitization, which requires rapid and complete solution exchange, as well as brief and controlled exposure times in patch clamp studies. Mr. Haedo and his team used several HEK-293 cell lines expressing homomeric nAChR α 7 subunits or heteromeric nAChR α 3 β 4 and performed automated patch clamp experiments using Patchliner and SyncroPatch96 with very high success rates. Ligand addition of Acetylcholine (ACh) and Nicotine on nAChR α 7 cell lines showed both accurate biophysical properties and proper pharmacology when compared with manual patch clamp literature values. In addition, preliminary data on the strong temperature dependence of allosteric modulation (several type 1 and type 2 PAMs) on nAChR α 7 and GABA_A receptors was shown. Temperature dependence of PAMs such as Diazepam was demonstrated for the first time on GABA_A receptors, suggesting a general temperature dependence on positive allosteric modulation of ligand-gated ion channels.

Next, Dr. Glenn Kirsch (Senior Director, ChanTest Corp) continued the discussion on GABA_A receptor screening studies. Due to the presence of subtypes of varying functionality, selectivity profiling of

GABA_A isoforms is a crucial step in the drug development. Dr. Kirsch discussed the development and optimization of automated electrophysiological characterizations of GABA_A receptors $\alpha 1$ through 5 co-expressed with $\beta 3$ and $\gamma 2$ subunits in HEK-293 cell lines using the IonWorks Barracuda platform. In particular, results for GABA response, diazepam response, and antagonist assays were shown, which were in agreement with literature and measurements from other platforms, including the PatchXPress. His team is currently working on the validation and automation of the assay.

In the next talk, Dr. Olaf Scheel (Chief Scientific Officer, CytoCentrics Bioscience GmbH) reported on virtual screening methods of compound cardiotoxicity. Dr. Scheel introduced an *in silico* prediction method as a cost-efficient and reliable high-throughput test for human *Ether-a-go-go*-related gene (hERG) liability. This method includes induced fit docking of potent hERG inhibitors and WaterMap calculations that can help identify the binding sites of drugs. Results from 29 test ligands were shown. The *in silico* ranking of IC₅₀ values were confirmed with whole-cell voltage-clamp experiments, which closely matched the predicted ranking. In addition, Dr. Scheel utilized the *in silico* prediction method to probe the mechanism of hERG inhibition. He concluded that the *in silico* model is a powerful approach for early safety screenings and that knowledge of binding mechanism of hERG inhibitors would help determine hERG blocking motifs in screening libraries. His team, in collaboration with Tyler Day from the company Schrödinger who developed the *in silico* methodology, is continuing to develop and further test the predictive power of this model.

Dr. Hervør Lykke Olsen (Scientific Service Manager, Sophion) followed up on the cardiotoxicology screening technology by reporting on HL-1 cells as a tool for validating current clamp recordings. HL-1 cells are immortalized atrial cardiomyocytes that beat spontaneously. Using this system, Dr. Olsen presented data on the electrophysiological properties and pharmacological profile of HL-1 cells through current clamp recordings with the QPatch HT, as compared with recordings from manual patch clamp data. In particular, data on action potential recordings were shown, with pharmacology affecting varying aspects of action potentials, including velocity, duration, early after depolarization, and stability. Dr. Olsen concluded that the results obtained by QPatch HT recordings were consistent with those obtained using manual current patch clamps as well as literature.

Next, Dr. Peter Miu (Field Applications Scientist, Molecular Devices, LLC) furthered the validation and optimization of the most recent addition to the automated platform, the IonWorks Barracuda system. He focused his presentation on the evaluation of the solution exchange rate and pharmacological performance, which are two important aspects especially for ligand-gated ion channel studies. Dr. Miu discussed the solution exchange rates using data on K_v1.3 channels in single-hole and population-patch-plate configurations, with the solution exchange rate of up to 40 and 80 ms, respectively. In addition, data on $\alpha 7$ and $\alpha 1$ nAChR and GABA_{1a} were shown to demonstrate the ability of capturing desensitization current kinetics for ligand-gated ion channels. Pharmacological studies were presented using hERG channel assays with multiple compound addition protocols, which exhibited highly reproducible IC₅₀ values compa-

ble to recordings from the IonWorks Quattro. Major strengths of the cross-plate compound plate design were discussed, which included improving the accuracy and shortening the assay run, thus preventing current rundown. Dr. Miu agreed with previous speakers that the IonWorks Barracuda has a high potential for being the platform of choice for early-stage, high-throughput screening for drug discovery.

Cardiac Safety and Toxicology

Ion channels are essential in the excitation, contraction, and repolarization of the heart. With cardiovascular effects being the leading cause of project termination and drug withdrawal in the pharmaceutical industry, understanding the effect that drugs have on ion channel function is critical.

Dr. Arthur “Buzz” Brown (Founder and Chief Scientific Officer, Chantest) opened the Cardiac Safety and Toxicology session by discussing drug-induced inhibition of hERG potassium channels and its utility in predicting delayed cardiac repolarization (DR), QT prolongation, and Torsade de Pointes (TdP). Dr. Brown questioned whether hERG studies alone could adequately determine drug risks. He described the Chantest model, which integrates multiple-ion channel effects (MICE), in an effort to better predict TdP with greater confidence than the hERG safety margin alone. By considering 39 drugs and their *in vitro* effect on hERG, Ca_v1.2, and Na_v1.5 channels, the corresponding IC₅₀ values were measured and compared with the effective therapeutic plasma concentration. It was found that some drugs which were previously considered torsadogenic because of their hERG IC₅₀ values were actually nontorsadogenic due to the offsetting block of the Ca_v1.2 and Na_v1.5 channels. By considering these MICE effects, many false positives and false negatives could be eliminated in order to provide a more accurate evaluation of the TdP risk. As a result, it was concluded that less emphasis should be placed on hERG inhibition alone.

Dr. Andrew Wojtovich (Postdoctoral Fellow, University of Rochester Medical Center) followed with studies into the identification of the mitochondrial large-conductance “big” K⁺ channel (mBK) in *Caenorhabditis elegans* and mice genetic models. This research is driven by the importance of the mBK in mediating anesthetic preconditioning (APC), a process that is key in protecting against ischemic-reperfusion (IR) injury. Dr. Wojtovich combined pharmacology with genetics to define specific targets, as they contribute to both mBK activity and APC. With current evidence pointing to the SLO1 gene as forming the mBK channel, a thallium fluorescent assay was used with the isolated mitochondria to assess the SLO isoforms that confer K⁺ transport across the mitochondrial inner membrane. Interestingly, the canonical Ca²⁺-activated BK channel SLO1 was dispensable for both mBK activity and APC in both models. Conversely, SLO2 was found to give rise to an mBK conductance that was necessary for APC. These findings suggest that SLO2 is a cytoprotective mitochondrial K⁺ channel and that it provides a novel therapeutic target to prevent pathologies associated with IR injury.

Next, Dr. Takashi Yoshinaga (Sr. Principal Scientist, Global Cardiovascular Assessment, Eisai Co. Ltd. [Japan]) discussed his research into the chronic effects of QT-prolonging drugs on the slow component of the delayed-rectifier potassium currents (IKs) using

KCNQ1/KCNE1 expressing CHO cells. Due to its regular application as a cholesterol-lowering drug and reported link to causing QT prolongation in clinical settings, Yoshinaga's team focused on probucol. Within 2 h of treatment, a reduction in IKs by 1 μ M was observed. After 24 h, the current was decreased by approximately 80%. After probucol treatment, it was discovered using western blotting that the multimeric complex of KCNQ1 proteins was reduced; however, the monomeric form was not. The results of this research suggest that chronic probucol treatment may contribute to QT prolongation in humans by decreasing the functional IKs channel complexes.

Dr. Gul Erdemli (Head of Ion Channel Group, Novartis Institutes for BioMedical Research, Inc.) focused her presentation on the detection of off-target human cardiac sodium channel (hNa_v1.5) inhibition. hNa_v1.5 plays a critical role in the action potential generation and propagation in the heart. Therefore, drug-induced inhibition can have serious implications for cardiac safety and may be considered a risk marker for drug candidates. Dr. Erdemli described a preclinical strategy for incorporating *in vitro*, *in vivo*, and *in silico* techniques as a means of integrated risk assessment for drug discovery. Results from 10 compounds (NVP1-10) were described, in which hNa_v1.5 liability was assessed by *in vitro* and *in vivo* studies. Dr. Erdemli emphasized the importance of an early detection and mitigation of hNa_v1.5 blockade in drug development.

Cardiac Function and Pharmacology

With cardiovascular disease being one of the leading causes of death in the United States, the role of ion channels in cardiac function and pharmacology is consistently a recurrent theme at the Retreat.

Dr. Tina Garyantes (CEO, MaxSAR Biopharma) discussed recent developments in the miniaturization of cardiac beating assays. She emphasized the importance of moving beyond hERG channel inhibition in drug risk assessment and the need to develop a practical approach to cost-effective cardiac toxicity studies. She showed literature data that whole cell measurements which consider the overall ion channel activity of induced pluripotent stem cell-derived cardiomyocytes are more predictive of clinical toxicity than measuring hERG activity alone. Furthermore, miniaturization of cardiac beating assays using DropArray technology from Curiox ensures cost-effective and rapid assessment. The DropArray is a "well-less" plate in which 384 drops of 2–4 μ L of beating, cardiac cells can be spotted. Dr. Garyantes demonstrated the validity of the system for long-term cell culturing, efficient washing, and high-quality imaging. She concluded that DropAssay is a robust and easy approach to a better predict cardiac risk for compounds.

Dr. Balwant Tuana (Professor, University of Ottawa) wrapped up the session by providing further insights into the regulation of calcium homeostasis by endogenous modulators and by pointing to a novel therapeutic target for calcium signaling. The talk centered on kinase anchoring protein (α KAP) and calmodulin-dependent protein kinase II (CaMKII). CaMKII are pivotal regulators of cardiac function and cardiac growth and are critical determinants of physiological versus pathological cardiac signaling. Dr. Tuana's results indicate that α KAP localizes in the SR membrane. Furthermore, he found that α KAP plays a critical role of binding to SERCA2a calcium transporters at the SR membrane, as well as recruiting CaMKII to the vicinity, which is crucial for regulating the activity of SERCA2a. Dr. Tuana also discovered the additional role of α KAP as the modulator of phospholamban at the SR membrane. In his ongoing research, Dr. Tuana is using a transgenic model to further decipher the mechanism *in vivo*, studying the effect of α KAP on important calcium release channels RyR and finding peptides that can disrupt the signaling pathway in cardiac cells.

CONCLUSION

This year, Aurora Biomed celebrated 10 years of hosting the Ion Channel Retreat with a wide range of speakers from both academia and industry presenting a variety of novel approaches for targeting ion channels for potential therapeutic solutions. Once again, the high caliber of speakers, strong leadership of the Scientific Advisory Board, and on-going support of the sponsors were key to the success of the Retreat. With novel screening technologies comprising the entire second day of talks, it is clear that automation is essential in maintaining low costs while improving efficacy and safety.

The speaker sessions stimulated detailed discussions that were further complemented by the variety of posters presented. Aurora would like to thank all the sponsors and attendees for their continued support in making the Retreat a success. The 11th annual Ion Channel Retreat will be held June 24–26, 2013, in Vancouver, British Columbia. Submissions for oral and poster presentations are now being accepted.

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