

# Meeting Report: The Seventh Annual Ion Channel Retreat Vancouver, Canada, June 29–July 1, 2009

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## ABSTRACT

Seven years ago, Aurora Biomed Inc. (Vancouver, BC) recognized the need to create a forum for scientific discourse spanning the spectrum of ion channel disciplines. Since then, researchers from both academia and industry have been invited each year to share their knowledge on the advancement of ion channel research and technology, drug discovery, and safety pharmacology. Aurora Biomed's 2009 Retreat continued this tradition by covering a variety of topics including Ion Channels as Disease and Pain Targets, TRP Ion Channels, Ion Channel Screening Technologies, Ion Channels in Safety Pharmacology, Structure & Function of Ion Channels, Ion Channels in Disease Pathology, and New Horizons in Life Sciences.

## INTRODUCTION

The recognized importance of ion channels in health and disease combined with the potential to develop new drugs targeting ion channels in a broad range of diseases has fueled the need to improve our understanding of their complex structure and function, as well as to develop more suitable screening technologies accounting for their complexity. To address such issues, Aurora Biomed relied on the guidance of its Scientific Advisory Board to assemble a program composed of speakers and poster presentations spanning a variety of subject matters relating to ion channel drug discovery and development and providing rich and diverse discussion topics. The 2009 Scientific Advisory Board was composed of Dr. Bernard Fermini (Pfizer Inc.), Dr. Georg C. Terstappen (Siena Biotech S.p.A.), Dr. Michael A. Dabrowski

(AstraZeneca), Dr. Shephali Trivedi (AstraZeneca Pharmaceuticals LP), Tony Priestley (Schering-Plough Research Institute), and Dr. Wei Zheng (NIH Chemical Genomics Center). The New Horizons Scientific Advisory Board included Dr. Craig T. January (Division of Cardiovascular Medicine & Co-founder: Cellular Dynamics International University of Wisconsin-Madison), Dr. Timothy J. Kamp (Division of Medicine and Physiology & Co-director Stem Cell and Regenerative Medicine Center, University of Wisconsin-Madison), and Dr. Umesh A. Patel (Millipore BioScience Division). Held in Vancouver, BC from June 29, 2009 to July 1, 2009, the seventh annual retreat welcomed scientists from a variety of academic and nonprofit research laboratories, alongside industrial laboratories representing sectors such as biotechnology and pharmaceutical industries.

## ION CHANNELS AS DISEASE TARGETS

As a follow-up to the discovery of channelopathies, recently an increasing number of human and animal diseases have been linked to defects in ion channel function and currently about 50 inherited ion channel disorders (“channelopathies”) are known (eg, long QT (LQT) syndrome). Because of their prevalence and the critical role they play in virtually all tissue types and organs, ion channels are also involved in a number of pathophysiological conditions such as cardiovascular, neurological and respiratory diseases, diabetes, and chronic pain conditions. As introduced by Georg C. Terstappen (Siena Biotech), ion channel drugs now make up 7% of the pharmaceutical market (over 10 billion dollars to date) ensuring their prevalence within the drug discovery community. However, the market for ion channel modulators remains under exploited as it has historically been challenging to identify quality leads within this target class because of the difficulty in screening ion channels in a cost-effective manner with validated functional screens that are relevant to clinical outcome at the throughput currently required by modern chemistry efforts.

## BRUGGER, KENNEDY, AND KING

Dr. Georg C. Terstappen next presented work on  $\alpha 7$  nicotinic acetylcholine receptors ( $\alpha 7$  nAChRs), rapidly desensitizing ligand-gated ion channels located in regions of the brain associated with cognition and sensory gating. He went on to discuss a novel  $\alpha 7$  nAChRs agonist WYE-103914 (SEN-34625) as a potential treatment for neurodegenerative and cognitive disorders such as Alzheimer's disease and schizophrenia. Dr. Terstappen showed that this agonist has the ability to enhance memory in rodent models, normalize sensory gating deficits in animals that are present in schizophrenics, and provide neuroprotective activity both *in vitro* and *in vivo*. Thus,  $\alpha 7$  nAChRs and specific agonists such as WYE-103914 (SEN-34625) may provide a novel approach for treating cognitive dysfunction.

Dr. Zhiyuan Li (Guangzhou Institute of Biomedicine and Health) continued the discussion of novel channel treatments by introducing 3 novel M2 ion channel antagonists. He explained that the M2 protein of influenza viruses forms a proton channel that is involved in pH regulation during infection but that many of the new viruses are not sensitive to the current M2 blockers. By establishing a stable M2 cell line expressing wild type and drug-resistant mutants, Dr. Li went on to show that although the M2 ion channel blocker, Amantadine, showed selective inhibition of the wild-type M2 proteins, cells expressing the M2 drug-resistant mutants were not inhibited. He presented 3 novel potent M2 ion channel antagonists that showed effective inhibition to both wild-type and mutant M2 channels. Thus, these blockers may be effective against the rapid development of drug resistance.

The discussions that followed by Dr. Heike Wulff (University of California) and Dr. Christine Beeton (Baylor College of Medicine) introduced both calcium-dependent and voltage-gated potassium channels. Dr. Wulff explained that calcium-activated potassium channels, KCa2.1–2.3 and KCa3.1, may represent effective drug targets for diseases such as ataxia, epilepsy, and hypertension. Furthermore, Dr. Wulff showed that her group has been able to exploit the synthetic route of Riluzole for the identification of 2 novel compounds: SKA-20 and SKA-31, both found to have good selectivity and pharmacokinetic properties for KCa2.1–2.3 and KCa3.1 channels. She showed that both compounds were effective as anticonvulsants and proposed that the activation of endothelial KCa3.1 channels may represent a potential novel antihypertensive mechanism.

The discussion of potassium channels was continued by Dr. Beeton, who presented the 2 types of potassium channels expressed by human natural killer (NK) cells. One is the calcium-activated potassium channel, KCa3.1, discussed by Dr. Wulff, whereas the other is Kv1.3. She showed data supporting activation of NK cells results in the release of proinflammatory cytokines and cytotoxicity. They found that adherent and nonadherent NK cells express

different ion channel collections and blocking Kv1.3 decreased the cytotoxicity. Therefore, she concluded that these channels may prove to be beneficial for selective targeting of different cell populations for treatment of autoimmune diseases.

To bring the session to a close, Dr. Luis Galiotta (Gaslini Institute) presented his work on the role of calcium-activated chloride channels (CaCC) in epithelial cells, as potential targets to correct defective chloride transport. His work looked at whether the transmembrane protein TMEM16A, a membrane protein known to be associated with CaCC activity (Caputo et al., *Science* 322:590–594,2008), forms the entire CaCC channel or whether there are other proteins involved. Using RNA extraction, microarray, and subsequent silencing via siRNA of candidate proteins, he found that inhibition of CaCC was found only after transfection of cells with siRNAs directed against TMEM16A. Similar results were seen when generating chloride currents in different cell types. Dr. Galiotta concluded that the activity of native CaCC was strongly dependent on TMEM16A expression with little contribution from other candidate proteins. TMEM16A might represent a new target for cystic fibrosis, asthma, and other diseases involving smooth muscle cell contraction.

## ION CHANNELS AS PAIN TARGETS

The involvement of ion channels in pain pathways has become an area of increased interest within the pharmaceutical industry. Pain caused by the persistent activation of ion channels is an area that is gaining more and more interest. However, even with the growing attention, the mechanisms of these pathways are not completely understood, and the issues of specificity and side effects still require investigation. For example, Dr. Alan Wickenden (Johnson and Johnson) explained that, with respect to Na<sup>+</sup> channel blockers, one of the major problems related to pain target research is finding a way to identify subtype selective channel blockers that could provide powerful analgesia without respiratory, central nervous system (CNS), or cardiac side effects. And as evidenced by the discussions that followed, it is clear that researchers are actively working to fill in these informational gaps.

Dr. Wickenden explained that Na<sup>+</sup> channels are responsible for action potential initiation and conduction. He highlighted the fact that current drugs lack selectivity and therefore can rarely be dosed to achieve concentrations that are 100% effective. The major goal of his study was to find a way to identify subtype selective Na<sup>+</sup> channel blockers that could provide powerful analgesia without respiratory, CNS, or cardiac side effects. He went on to explain that standard approaches did not consider flaws such as the fact that the binding site for anesthesia is highly promiscuous and highly homogeneous. Dr. Wickenden highlighted that this

leads to high hit rates in compound screening that overwhelms the capacity of secondary screening assays. He showed that allosteric modulator sites may offer potential for increased subtype selectivity as they target nonconserved regions remote from the pore and suggested that radiolabeled peptide ligands may represent a bright future for Na<sup>+</sup> channel research in drug discovery.

Dr. Andreas Jeromin (Allen Institute for Brain Science) discussed the role of the Kv4.2 pore-forming subunit in models of epilepsy and pain using total internal reflectance microscopy with a very high resolution. He monitored the insertion of Kv4.2 and mutants into the plasma membrane of cultured hippocampal neurons and studied the induction of plasticity in neurons in culture. Dr. Jeromin showed that they were able to identify molecular mechanisms of dendrite targeting of Kv4.2 channels specifically interacting with SAP97 and PSD via the C terminus.

### TRP ION CHANNELS—REGULATION AND LINKS TO PAIN AND DISEASE

It is thought that a focus on therapies that target ion channels responding to thermal, mechanical, and chemical stimuli may allow the treatment of patients who experience inflammatory or neuropathic pain. Transient receptor potential (TRP) channels are ligand-gated calcium permeable ion channels that contribute to the response of such stimuli. TRP channels are transient receptor cation channels that are seen to play a large role in tissue regulation. As introduced by Dr. Wei Zheng (NIH), 28 mammalian TRP channels have been identified, many of which have pathways, mechanisms, and structures that are relatively unknown. Notably, this is the first year where the discussion of the regulation and role of TRP channels in pain and disease has been extensive enough to require its own section for discussion.

Dr. Roger O'Neil (University of Texas Health Science Center) opened the discussion by presenting his work on TRPV4 channels. These channels have been implicated in various diseases including neuropathic pain and asthma. His work involved monitoring the TRPV4 trafficking in loss-of-function Kv4.2-mutant HeLa cells by confocal microscopy and total internal reflection fluorescence (TIRF). This examination revealed channels moving in and out of cell attachment sites or fibrillar processes. Fixed cells were shown to exhibit TRPV4 localization in the membrane and cytoplasm. Also, by activating the channel with 4 $\alpha$ -phorbol 12,13-didecanoate (4 $\alpha$ -PDD), Dr. O'Neil showed an activation-dependent movement of TRPV4 within the cytoskeleton between microtubules and actin. He concluded by stating that TRPV4 channels appear to assemble prior to insertion into the plasma membrane.

Phosphoinositides (PI) have been shown to potentiate TRPV1 channel gating and calcium regulation. Dr. Sharona Gordon's (University of Washington) presentation explored the

identification of the specific PIs involved by determining if the degradation of PI(4,5)P(2) or PIP2 was enough to inhibit TRPV1 activation. This was done by monitoring whole cells and excised patches. Sequestration of PIP2 by polylysine (also known as PolyK) in excised patches inhibited TRPV1 when exposed to capsaicin. Using cells transfected with a rapamycin-inducible lipid phosphatase, rapamycin treatment produced decreases in PIP2 and likewise inhibited TRPV1 current. Her studies then turned to using recombinant pleckstrin homology (PH) domains to selectively sequester PIP2 and confirm that it was the endogenous phosphoinositide bound to TRPV1 and modulating its current. The discovery that PIP2 is the endogenous PI regulating TRPV1 indicates a promising focus for therapeutic research.

Dr. John "Jack" Stewart (BioProspecting NB Inc.) continued the discussion of TRPV channels as calcium regulators. His discussion centered on TRPV6 that plays a central role in regulating entry and exit of calcium into cells during many body processes. While it functions to maintain calcium homeostasis, it has also been correlated with tumor development in prostate, breast, thyroid, colon, ovary, and pancreatic tumors. TRPV6 has been shown to mediate the downstream activation of nuclear factor-activated T-cell transcription (NFAT), which appears to enhance survival of cancer cells. Dr. Stewart's group isolated a peptide from the saliva of the Eastern North American shrew that severely inhibits calcium movement through TRPV6 channels. The peptide they isolated (Soricidin) has been shown to shut down the survival mechanism of these cells and induces apoptosis *in vitro* and *in vivo*. As the peptide appears to target only TRPV6 expressing cancerous cells, healthy cells are not affected. Dr. Stewart presented this new peptide as a potential novel anticancer agent.

### ION CHANNEL SCREENING TECHNOLOGIES

As noted by one of this section's speakers, Dr. Michael Dabrowski (AstraZeneca CNS & Pain), things truly have come along way in the 65 years of ion channel research. From the introduction of the voltage clamp technique in 1949 to high-throughput screening technologies of the industry today, the massive advancements are hard to ignore. But even so, discussions regarding the ever-changing technologies continue en masse. Researchers are constantly refining assays, developing novel, more efficient screening methods, and improving existing methods. In the following presentations, researchers discussed the importance of target validation, lead generation, and seamless assays.

The discussion was initiated by Dr. Greg Kaczorowski (University of Medicine and Dentistry of New Jersey and Robert Wood Johnson Medical School) who began his presentation by pointing out that potency, selectivity, and appropriateness are all integral factors in identifying leads for drug development.

Lead identification is a rate-limiting step in the process of ion channel drug discovery and it is important to have dependable high-throughput screens and translatability of *in vivo* animal models. Drug development typically has a very high failure rate but a variety of screening methods have been developed to speed up validation of the compound to clinical candidate status. Dr. Kaczorowski discussed state-dependent binding of novel modulators. His work suggested that blocking a channel based on its conformational state will determine if the compound is a potentially safe therapeutic agent since strong binding to the inactivated state vs. resting is preferred. He went on to explain how detection of active compounds could be monitored by fluorescent dyes as the addition of active compound changed membrane potential as determined using voltage-sensitive dyes. This appears to be a promising screening strategy for determining the difference between state-independent vs. state-dependent blockers, and in some cases, defining the difference between a novel therapeutic candidate and a poison.

To continue the discussion of screening technologies and assay development, Dr. Dabrowski introduced the Global Ion Channel Initiative launched by AstraZeneca in 2007. He discussed the challenges of drug discovery with 4 components of its dependency: (1) hit and lead generation (dependent on type of screening technology, compound generation, and structural chemistry), (2) expression and subunit composition, (3) ion channel target identification and validation, and (4) selectivity of the screen. He pointed out that after sequencing the structure of a targeted channel like TRPV1 they were able to determine that a large portion of the channel's sequence was not conserved between rats and humans thus the use of foreign species' channels as targets for drug validation was not a reliably predictive source of data. He discussed the usefulness of voltage-dependent vs. usage-dependent block of channels and how each method contributed differently depending on the channel in question. He then compared 2 versions of the IonWorks systems, the HT and Quattro, that have 1 hole per patch well vs. 64 holes per patch well, respectively, and highlighted that these systems could not look at fluidics and voltage at the same time and did not attain gigaohm seals. He added that the QPatch system allowed the creation of the gigaohm seals and offered other benefits but at high cost per data point and required significant maintenance. His instrument of choice for their initiative has been the DYNAFLOW HT by Celectricon that has superior microfluidics and online control with very high-throughput, low-cost, and reproducible, reliable data. He concluded that they have been using the lentiviral toolbox for studying expression, which is useful for infecting non-dividing cells for a quick infection of new cell lines and multiple gene transfer with no gene silencing.

Dr. Wei Zheng (NIH) discussed the importance of  $K^+$  channels as targets in drug safety assessment and has developed an assay for measuring these channels in 1,536-well plate format. He described the principle of the thallium flux assay using the FluxOR™ thallium detection assay. This assay uses a fluorescent sensor dye that is quenched by thallium ( $Tl^+$ ) tracer ions, which reflects the movement of the ions from the external medium and the activity of ion channels that transport this ion. The assay incorporates a Red-40 non-cell-permeable quencher that eliminates an extra wash step. The  $IC_{50}$ s of patch clamp vs.  $Tl$ -flux for 10 known hERG inhibitors were compared and shown to correlate well.  $Tl$ -flux assays in 1,536-well format appeared to be suitable for primary screens and profiling for  $K^+$  channels. Also, Dr. Zheng discussed NIH grant information for HTS assay submission and development as well as their associated costs and deadlines.

Dr. Victor Uebele (Merck) discussed how T-type calcium channels play a role in several ailments including epilepsy, obesity, pain, Parkinson's disease, and others. He explained that other voltage-gated calcium channels exhibit state- and rate-dependent inhibition depending on the location with which the compound interacts. FLIPR was used as a high-throughput screen to monitor the potency and state-dependent properties of T-type antagonists (TTAs). TTA-A1, one of these novel antagonists, was radiolabeled and used on HEK293 cell line expressing one of these channels,  $CaV3.3$ . A second novel antagonist, TTA-Q4, demonstrated increased affinity in a saturable manner, slowed dissociation, and enhanced amide radioligand binding. FLIPR analysis showed these compounds to be state-dependent with positive allosteric modulation while the state-independent compound TTA-P1 did not modulate either TTA-A1 or TTA-Q4. Further studies were performed on genetic rat models of absence epilepsy whereby the compounds' effects were prolonged. His experiments demonstrated that such modulators could be distinctly categorized using high-throughput assays and the combined information provided from their binding sites and electrophysiological properties.

Dr. Sikander Gill (Aurora Biomed) spoke on the advantages of combining flux assays with atomic absorption spectroscopy (AAS) for primary and secondary screenings of ion channel targets. Over the past year, novel assays have been developed for pharmacologically important ion channel targets: acid-sensing ion channels (ASICs), stretch-activated channels (SACs),  $Na^+-K^+$  ATPases and purifying proteins in synthetic vesicles (liposomes). ASICs, which are  $Na^+$ -selective channels, are activated by tissue acidosis that is caused by a local pH drop often seen to occur in chronic pain conditions such as inflammation, arthritis, cancer, and others. The assay uses  $LiCl$  in the activation buffer as measurement of the tracer ion  $Li^+$  is used as an indicator of channel activation elicited by a change in pH.  $Li^+$  is measured by AAS.

The principle of this assay is similar to those developed for SACs and Na<sup>+</sup>-K<sup>+</sup> ATPase except in this case Rb<sup>+</sup> is being used as the tracer ion for the movement of K<sup>+</sup> through both of these membrane proteins (Na<sup>+</sup> and K<sup>+</sup> are exchanged across the ATPase transporter). SACs affect electrical activity in cardiac myocytes, while cardiac myocytes in mouse embryonic stem cells were used to study ion movement in the ATPases. This AAS-based method for high-throughput screening of such channels can handle up to 60,000 data points in 8 h using Aurora Biomed's ICR 12000.

Dr. Søren Friis (Sophion Bioscience) discussed the QPatch HTX produced by Sophion Bioscience, which is the newest automated patch-clamp system available from this company and can be used to screen both voltage-gated and ligand-gated channels. The silicon chip is the plate used to form gigaseals with cells using integrated microfluidic technology. Each measurement site can record from multiple cells, which solves the problem of patching nonexpressive cells and having to repeat experiments. This system can provide 7,000 data points per day and can run in single- or multi-hole mode depending on whether compound profiling or screening is selected. There was some concern that the fluid exchange time of the system would not be consistent with the QPatch HTX as it was in the QPatch HT due to the wider separation of patch holes in the HTX for the multiple holes it requires. The HTX was shown to maintain its rise times for all tested ligand-gated ion channels. Dr. Friis presented data on several receptors and their agonists and antagonists used to validate this system and concluded that throughput was increased with the HTX vs. the HT while fidelity was maintained.

Ion channels are necessary for the detection of all types of taste stimuli. Dr. Bryan Moyer's presentation (Senomyx Inc.) focused on identifying some of these targets of taste stimuli in order to potentially make foods and beverages more appealing and nutritious. The IonWorks Quattro<sup>®</sup> was used to identify activators and blockers of taste cell ion channels in a screen with 300,000 compounds. Senomyx used cloned human taste receptors for their screen and have isolated a Na<sup>+</sup> leak channel (confidential target) as the main focus of their current research using NMDG (*N*-methyl-D-glucamine) to artificially block this channel. Confirmed hits were validated using OpusXpress<sup>®</sup> and manual patch clamping. Identification of lead molecules for this target therefore was shown to be possible with the IonWorks high-throughput screening system.

Dr. Ralf Kettenhofen (Axiogenesis AG) discussed how 3 types of automated patch-clamp systems (the PatchXpress (MDS-AT), the QPatch (Sophion), and the Port-a-Patch (Nanion)) were used to validate mouse embryonic stem cell-derived Cor.At.<sup>™</sup> cardiomyocytes. These cells were ideal for validation on these systems as they are a homogeneous cell population and possess spherical cell

morphology. Na<sup>+</sup>, Ca<sup>2+</sup> L-type, and K<sup>+</sup> conductances, as well as their dose-response relationship, were recorded on all 3 systems; current-clamp recordings were achieved using the Port-a-Patch. These cells may prove useful for safety pharmacology studies as the erg channels are reversibly blocked with dofetilide. The pharmacology of Na<sup>+</sup>-K<sup>+</sup> ATPases endogenously expressed in these cells and its modulators were validated using the Rubidium flux assay/atomic absorption spectroscopy automated system, the ICR 8000 (Aurora Biomed) and discovered to offer useful data from this assay as well. The cells were found to be very predictive in pharmacological studies when validated by both automated and patch-clamp methods.

### NEW HORIZONS IN LIFE SCIENCES

This topic was added to the Ion Channel Retreat repertoire this year and offered a novel expansion in the field of ion channels and drug development research. 454 Life Sciences has provided the means to sequence entire genomes in a matter of days and has been used to map genetic variation of ion channels within species and how this might predict disease phenotypes. Stem cell research has been directed at providing new platforms for a reliable source of easily maintained and renewable pluripotent cell lines.

Dr. Richard McCombie's group (Cold Spring Harbour Laboratory) has been researching proteins within synapses and sequenced the genes that make up these proteins to determine how variations and associated phenotypes might contribute to neurologic disorders such as schizophrenia and bipolar disorder. Their research at Cold Spring Harbour Laboratory utilizes 11 Next Generation Sequencers from Illumina to perform sequencing by synthesis for their genome association studies. This technology has allowed them to sequence the genomes of entire families of patients with these disorders and they have recently begun analyses of transcripts and RNA. This high-level analysis of variant information has made it possible to link these data with the phenotype or disease of patients. Dr. McCombie predicted that the cost of sequencing entire genomes based on the speed due to sequencing technology improvements would be less than US\$10,000 in the very near future and that variant analysis informatics would soon take over as the rate-limiting step for the entire process.

Dr. Steven Stice (University of Georgia) discussed the importance of embryonic stem cells (ESC) as a source of pluripotent cells that consistently differentiate into embryonic precursors for all tissues and therefore may provide a reliable starting source of research material for drug discovery applications. Neural cells appear to be the most proliferative from this group and the most useful for working with potential drug targets due to their stability and ease of maintenance. He discussed the use of their adherent monolayer stem cell platform technology in a variety

of research applications including cellular model studies, high content screening, developmental and RNAi studies, and genetic manipulation. His monolayer platform ensured high-quality assay results during drug screening as the cells were uniformly exposed to the test compound and did not require serum or a feeder layer for maintenance. Their neural progenitor cells were also able to be directed to differentiate into any cell type of the CNS at high density for all types of assays and toxicity screening.

Dr. Clive Glover (StemCell Technologies) exemplified the importance of standardizing development of human embryonic and inducing pluripotent stem cells for consistency, safety, and reproducibility reasons. He introduced a newly developed media (mTeSR™) designed specifically for maintenance of karyotype and pluripotency of these cells. Another new product, the AggreWell™, used microwells to efficiently control differentiation of cells and produced uniform-sized embryoid bodies. These platforms allowed for stem cells to be maintained as uniform embryoid bodies from a single progenitor.

Dr. Tim Kamp (University of Wisconsin/Cellular Dynamics Inc.) closed this session by introducing novel applications of embryonic stem cells in cardiac safety. Since ion channels proteins of the heart vary greatly between species, it may be more applicable to use *in vitro* models for screening and pharmacology purposes. He then elaborated on the use of stem cell-derived cardiomyocytes for such purposes. These cells can be used for cardiotoxicity profiling of compounds and are consistent enough to be used in drug screening. He explained that these cells would soon be derived exclusively from human-induced pluripotent stem cells (iPSCs) from genetically diverse samples, thus allowing a more representative population sample to be examined in assays. It has generally been difficult to differentiate stem cells into cardiomyocytes, but lately this differentiation has been enhanced with the use of genetic selection to produce cardiomyocytes with a purity of >90%. These cells have been confirmed to respond correctly to electrical stimulation and QT-prolonging agents, which indicates that they have the potential to be useful for toxicity screening and drug development.

### SAFETY PHARMACOLOGY

One of the major reasons for withdrawal of marketed drugs or drug label revision is drug-induced cardiac toxicity, so this might be an important liability of compounds under development. New potential drug molecules are required to pass cardiac toxicity testing according to the ICH S7B guidelines, which are designed to collect critical data for the approval process. Talks in this year's safety pharmacology section focused on refining methods for preliminary assessments of hERG and further cardiac liability, the possibility of using *in silico* and *in vivo* characterization, and

approaches to identify further potentially dangerous inhibitory interactions.

To begin the discussion, Dr. Craig January (University of Wisconsin/Cellular Dynamics Inc.) introduced hERG potassium channels and the ongoing research into the molecular mechanisms responsible for the hERG-associated inherited long QT syndrome (LQTS) and drug-induced or acquired LQTS. Genetic mutations are thought to be the cause of protein-trafficking abnormalities in the majority of inherited LQTS cases. For drug-induced LQTS, many drugs block hERG channel pore (direct block) but a few also disrupt drug trafficking (indirect block). Through observations of the heterologous expression in noncardiac and cardiomyocyte systems, Dr. January studied channels carrying human mutations, most of which were single amino acid substitutions, and showed that the channels either failed to traffic normally, had abnormal gating, or both. He showed that most of the mutations resulted in trafficking-deficient channels. Dr. January went on to explain that they found they could rescue the mutant channels either by using drugs that bind to the channel or by lowering the temperature in culture, which could induce the channels to traffic.

Dr. Bernard Fermini (Pfizer) took the discussion of hERG channels and QT prolongation one step further by highlighting the process his group took to identify compounds that may inhibit hERG, possibly leading to QT prolongation. He proceeded to introduce the correlation between blocking Na<sup>+</sup> channels (human cardiac Nav1.5 channel) and prolonging the QRS component of the electrocardiogram. His group looked at different Na<sup>+</sup> channel blockers and the ratio of free exposure where QRS prolongation was observed in various *in vivo* models, including dogs and nonhuman primates from which blood samples were obtained to define the pharmacokinetic–pharmacodynamic (PKPD) profile of a series of compounds. The results showed changes in QRS duration at concentrations below that of Nav1.5 IC<sub>50</sub> values, suggesting that small inhibition of Nav1.5 current may lead to significant increases in QRS duration, establishing QRS widening as a growing concern in the pharmaceutical industry. By applying the knowledge gained from previous data on drug-induced LQTS, a screening strategy was put forward to address QRS prolongation.

Dr. Stephen Hess (Millipore) went on to propose screening cardiac channels beyond hERG as a means of determining the safety of drug molecules early in the development process. Evidence exists suggesting that the QT interval effect of hERG block can be modified by drug effects at other cardiac ion channels and the use of primary animal cells and tissues may not be a reliable source of useful preclinical test data to represent the human heart. Other channels in the human heart include Kv1.5, Nav1.5, CaV1.2, Kv4.3/KChIP2, Kv7.1/KCNE1, Kir2.1, and HCN4. Dr. Hess proposed that the use of his newly developed cardiac profiler assays could

provide more predictive cardiac toxicology screening data at earlier stages of drug development and at reduced cost.

Dr. Clemens Möller (Evotec) discussed *in silico* models for predicting hERG liabilities. He explained that histamine receptor 3 (H3) antagonists are known to have a propensity for hERG block. Möller's work looked at finding new ways to predict potential hERG liability. Electrophysiological hERG measurements of a subset of compounds from medicinal chemistry iterations were used to establish a structure–activity relationship (SAR). Fifty-seven compounds were tested in manual and automated patch-clamp assays to assess the reliability of the data obtained on the automated device. These data were then compared to the *in silico* models of the channel and a poor correlation was found between electrophysiological results and the 2D *in silico* prediction, while an excellent correlation was found between electrophysiological results and 3D *in silico* prediction. He explained that this may be because the 3D *in silico* prediction took into account the predicted electrostatic interaction of compounds with regions of the hERG inner pore. The correlation also showed that the electrostatic interaction could play a role in hERG binding. Thus, he concluded that the 3D computational model could play an important role in identifying hERG liabilities in H3 targets.

Dr. Paul Li (Simon Fraser University) discussed the application of real-time detection of calcium channel events of single cells for drug efficacy and cardiac toxicity evaluations. He explained that by using microfluidics, the dynamic intracellular calcium ion mobilization could be measured using fluorescent indicators. Dr. Li treated both leukemia cells and single rabbit cardiomyocytes with various chemical reagents such as daunorubicin (DNR), cardiotonic agents (caffeine), ionophore (IM), and herbal ingredients from licorice (IQ). Observations of the dynamic intracellular calcium ion mobilization showed that the all but the IQ had various cytotoxic effects on both cells. The IQ showed cytotoxic effects on only the leukemia cells; therefore, Dr. Li concluded that microfluidics could be a useful tool for *in vitro* cell-based evaluations of drug efficacy and toxicity on single cells.

## STRUCTURE AND FUNCTION OF ION CHANNELS

Research discussed over the course of the ion channel retreat reinforced the idea that ion channels may be classified as belonging to several families according to their structural similarity. By understanding the physical nature of ion channels, researchers will better understand how they work, and will gain a better understanding of how these structure–function relationships work from a molecular level. Dr. Damian Wheeler (Stanford University) and his colleagues have been researching the role of  $\text{Ca}^{2+}$  channels on control of transcription events and how they contribute to learning and memory processes. L-type channels (CaV1 family)

appear to signal transcription preferentially over CaV2 channels by several mechanisms: (1) they appear to activate more negative potentials, (2) CaV1 channels signal more locally than CaV2, and (3) mitochondria down-regulate CaV2 signaling of transcription. This mitochondrial buffering may affect other important cellular processes. They have also shown that signaling by the channels is regulated by 2 parallel pathways. Depolarization will cause local  $\text{Ca}^{2+}$  influx to signal the transcription factor CREB and this signaling is controlled by both the  $\text{Ca}^{2+}$  influx and the channel open-state probability,  $P_o$ . His results illustrated the mechanisms of excitation–transcription coupling and may provide insight on other cellular processes that could be targets of future drug development studies.

Dr. Gerhard Meissner (University of North Carolina, Chapel Hill) discussed cardiac ryanodine receptor channels. Ryanodine receptors (RyRs) are made up of 4 peptide subunits with one binding site per unit to bind apoCaM or CaCaM ( $\text{Ca}^{2+}$  free and  $\text{Ca}^{2+}$ -bound forms of calmodulin): release and sequestration of this ion from the sarco/endoplasmic reticulum regulates contraction and relaxation of the heart. Mutagenesis studies conducted in his lab using mice expressing isoforms of calmodulin, which were no longer able to bind  $\text{Ca}^{2+}$ , resulted in early postnatal death. The calmodulin-binding site was also highly conserved across all the mammalian isoforms of RyRs. Inhibition of the RyR2 channel appeared to occur following binding of calmodulin at low concentrations of  $\text{Ca}^{2+}$ . Calmodulin binding appeared to result in longer duration closings and lower probability of openings of the RyR2 channel.

Chloride channels (ClC) are also important for many physiological processes in a variety of species and can serve as potential therapeutic targets for many afflictions. Dr. Meritt Maduke (Stanford University School of Medicine) has been researching small-molecule inhibitors and their ability to function as conformational state attenuators as well but useful results are lacking. She used selected crystallization methods of visualization of the protein structure of ClC-ec1 homologs in prokaryotes to determine potential sites of interaction for some of the newest inhibitors of ClC channels to date.

Dr. Raad Nashmi (University of Victoria) concluded the section with a discussion regarding his group's attempt to identify the neuronal changes that occur as a result of chronic nicotine exposure. They engineered knock-in mice to express yellow fluorescent protein-tagged  $\alpha 4$  nicotinic acetylcholine receptors (nAChRs) and exposed these mice to varying levels of nicotine. Their results did not indicate a change in the number of the  $\alpha 4$  receptors in neurons in brain areas thought to be important to nicotine addiction. However, these receptors were found to be more responsive in GABAergic neurons. They also found that up-regulated receptors

## BRUGGER, KENNEDY, AND KING

in the medial perforant path of the hippocampus reflected sensitization of synaptic transmission in the forebrain by exposure to chronic nicotine.

### CONCLUSION

This year's retreat covered several new subjects from the list of topics researched and discussed by leaders in the field of ion channels and drug discovery. The previous focus on the link between ion channels to pain and disease has shifted slightly to the connection between TRP channels and these afflictions. Some new contributions to ion channel research include pyrosequencing with 454 Life Sciences technology, which appears to be contributing significant data toward mapping of genetic ion channel variation and disease outcomes. New stem cell platforms are being developed that will potentially offer an efficient, renewable, and maintainable source of pluripotent cells expressing ion channels for screening and toxicity testing. High-throughput screening automation platforms have dramatically improved over the last few years and now offer a variety of reliable semi- or fully automated solutions to perform screens once thought to be possible

only by tedious and low-throughput manual patch clamping. These methods promise to significantly reduce, if not eliminate, the bottlenecks associated with compound screening at ion channels and should lead to increased efficiency and output, and perhaps lowering costs associated with bringing drugs to market.

Aurora Biomed's Ion Channel Retreat is a yearly event that began in 2002 and has consistently provided a platform for researchers to congregate, to share ideas, and to potentially discuss future collaborations and directions. We thank you for your continued support of this conference, and we welcome submissions for the eighth annual Ion Channel Retreat to be held June 28th through June 30th, 2010, in Vancouver, BC.

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