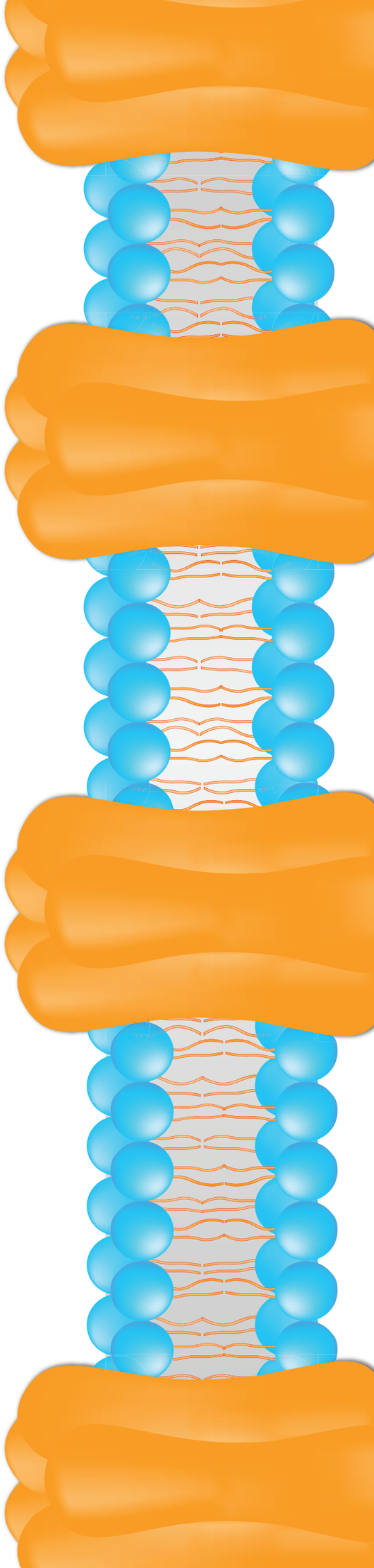
SOPHION BIOSCIENCE

ION CHANNEL MODULATION SYMPOSIUM 2019

June 19-29, 2019



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Agenda - 19th June, 2019

08.00	Registration - Tea/Coffee
09.00	Welcome Remarks
	Session 1 - Chair: Professor Stephen Tucker - University of Oxford, UK
09.15	Dr Guillaume Sandoz - University of Côte d'Azur, France *Migraine-associated TRESK mutations increase neuronal excitability through alternative translation initiation and inhibition of TREK*
09.40	Dr Marcus Schewe - Christian-Albrechts-Universität zu Kiel, Germany *A pharmacological master key mechanism that unlocks the selectivity filter gate in K^+ channels*
10.05	Professor Sarosh Irani - University of Oxford, UK *Autoantibodies in the human brain: how immunology upsets neurons*
10.30	Tea/Coffee, Exhibits and Posters
11.15	Professor Sylvie Ducki - Institut de Chimie de Clermont-Ferrand, France *TREK-1 activation to solve the opioid crisis?*
11.40	Professor Slav Bagriantsev - Yale University, USA *Cellular and molecular basis of mechanosensory adaptations in tactile specialist birds*
12.05	Dr David Hackos - Genentech, USA *Structural basis of toxin binding to voltage-gated sodium channels and what it teaches us about voltage-gating mechanisms*
12.30	Lunch
	Session 2 - Chair: Professor David Wyllie - Edinburgh University, UK
14.00	Dr Paul Wright - Lifearc, UK *A 'Target-class' approach to identifying novel activators of K2P channels*
14.25	Professor Fusao Kato - Jikei University, Japan *Molecular conversion of presynaptic P2X receptor subtype at brainstem synapse*
14.50	Dr Sung-Young Kim - Daewoong Pharmaceutical Co., Ltd., Korea *Drug development strategies of $Na_V1.7$ blocker*
15.15	Tea/Coffee, Exhibits and Posters
16.00	Professor Jack Mellor - Bristol University, UK *SK channel regulation of synaptic plasticity*
16.25	Professor Isabel Pérez-Otaño - Alicante Institute of Neuroscience, Spain *Juvenile NMDA receptors containing the GluN3A subunit: gate keepers of synapse plasticity, cognition and brain disease*
16.50	Professor Thomas Jentsch - Max-Delbrueck-Center Berlin, Germany *The CIC-2 chloride channel and its role in aldosterone secretion*
17.15	Wrap Up
17.30	Drinks Reception in the Scholar's Garden
18.30	Dinner in the Great Hall of Clare College

Agenda - 20th June, 2019

08.00	Tea/Coffee
09.00	Welcome Remarks
	Session 3 - Chair: Professor David Beech - Leeds University, UK
09.05	Professor Stefan Feske - New York University, USA *Ion channels in immunity: CRAC channels and beyond*
09.30	Professor Dr Marc Freichel - University of Heidelberg, Germany *Regulating pathological cardiac remodeling via TRPC channels and new players in endo-lysosomal Ca^{2+} signaling*
09.55	Professor Insuk So - Seoul National University, Korea *TRPC1 as a negative regulator for TRPC4 and TRPC5 channels*
10.20	Tea/Coffee, Exhibits and Posters
11.20	Professor Alex Sobolevsky - Columbia University, USA *Structural and functional studies of vanilloid subtype TRP channels*
11.45	Professor Thomas Voets - KU Leuven, Belgium *Temperature-sensitive TRP channels as drug targets*
12.10	Professor Yoshihiro Kubo - National Institute for Physiological Sciences, Japan *Phosphoinositides modulate the voltage dependence in two-pore Na^+ channel 3 (TPC3)*
12.35	Lunch
	Session 4 - Chair: Professor Annette Dolphin - University College London, UK
14.00	Professor Mala Shah - University College London, UK *Function and modulation of axonal K_V7 channels in hippocampal neurons*
14.25	Professor Fredrik Elinder - Linköping University, Sweden *Resin-acid derivatives open potassium channels via the voltage-sensor domain – a putative anti-seizure action*
14.50	Dr Paul Miller - University of Cambridge, UK *Insights into understanding and developing modulators of $GABA_A$ receptors*
15.15	Professor Dimitri Kullmann - University College London, UK *Ion channel gene therapy for refractory epilepsy*
15.40	Wrap Up

Biographies - Advisory board

David Beech - Leeds University, UK

David Beech graduated in Pharmacology from the University of Manchester UK in 1985 before PhD study with Thomas Bolton at St George's Hospital Medical School London and postdoctoral training with Bertil Hille at the University of Washington Seattle USA. In 1992 he established an independent research group at the University of Leeds UK, funded initially by a Wellcome Trust Postdoctoral Career Development Fellowship and then a full university professorship since 2000, moving from the Faculty of Biological Sciences to the Faculty of Medicine and Health in 2013. His research is focussed on calcium-permeable non-selective cationic channels of mammalian cells – their mechanisms, roles and potential as new therapeutic targets. He is particularly interested in the idea that the channels importantly sense physical and chemical factors to regulate cardiovascular and metabolic health. He has trained 74 postgraduate and postdoctoral research scientists, published 150 peer-reviewed articles (including 2 in Nature and 4 in Nature sister journals) and delivered 157 invited lectures worldwide. He was elected to Fellowship of the Academy of Medical Sciences in 2013 and became a Wellcome Trust Investigator in 2016 and British Heart Foundation Programme Grant Holder in 2018. Since 2016 he has been Director of the Leeds Institute of Cardiovascular and Metabolic Medicine, a research and teaching organisation of over 200 staff in the School of Medicine at Leeds. He founded and continues to direct the British Heart Foundation 4-Year PhD Programme in Cardiovascular Disease and Diabetes and the Multidisciplinary Cardiovascular Research Centre, a pan-university / teaching hospital structure for all cardiovascular research in the Leeds region.

Professor Annette C. Dolphin - University College London, UK

Annette Dolphin received her BA in Natural Sciences (Biochemistry) from the University of Oxford and her PhD from University of London, Institute of Psychiatry. She then held postdoctoral fellowships at the College de France in Paris, and at Yale University, before returning to the UK to the National Institute for Medical Research, London; followed by a lectureship in the Pharmacology Department of St. George's Hospital Medical School, London University. She was appointed Chair of the Department of Pharmacology at Royal Free Hospital School of Medicine, London University in 1990, and moved to University College London in 1997. She is a Professor of Pharmacology in the Department of Neuroscience, Physiology and Pharmacology at UCL. She was elected to the Academy of Medical Sciences in 1999, and the Royal Society in 2015. She is a Wellcome Trust Senior Investigator and held a Royal Society Leverhulme Trust Senior Research Fellowship (2016-17).

Stephen Tucker - Oxford University, UK

Stephen Tucker is a Professor of Biophysics in the Clarendon Laboratory at the University of Oxford, and also Director of the Wellcome Trust PhD programme in Ion Channels and Disease. After studying Biochemistry at Oxford he did his PhD at the Institute of Molecular Medicine at the John Radcliffe Hospital studying the CFTR chloride channel. After this, he went to the Vollum Institute, Oregon USA for two years as a Wellcome Trust International Prize Travelling scientist, and in 1996 he returned to Oxford as a Wellcome Trust Career Development Fellow where he worked closely with Prof Dame Frances Ashcroft, FRS on the ATP-sensitive K+ channel and other inwardly-rectifying K+ channels. In 2000 he was awarded a Royal Society University Research Fellow in the Department of Physiology, and in 2008 he was appointed to a University Lecturership in the Department of Physics. In 2015 he was made a Professor of Biophysics in the Department of Physics and is currently a fellow of Green Templeton College, Oxford. The Tucker lab employs a wide range of structural, functional and computational approaches to study ion channels, and current work is focussed on the Two-Pore domain (K2P) family of potassium channels.

David Wyllie - Edinburgh University, UK

My long-standing research interest is in ligand-gated ion channels (LGICs) – specialized pore-forming membrane proteins that are activated by neurotransmitters during 'fast' chemical synaptic transmission. In particular, my lab studies LGICs activated by L-glutamate – the major excitatory neurotransmitter in the mammalian brain. Although glutamate activates several different classes of LGIC one, in particular, the N-methyl-D-aspartate receptor (NMDAR) has been a major focus for our research. Through electrophysiological studies, my lab has contributed significantly to our understanding of the structure-function properties and physiological roles of the various subtypes of NMDARs. NMDARs play pivotal roles in both normal and abnormal brain function. In early life, for instance, they ensure that the correct wiring pattern is laid down in the developing brain. Furthermore, activation of NMDARs is required to learn certain tasks and store memories. However, both over- and under-activation of NMDARs can be deleterious for normal brain function. For example, during a stroke excessive activation of NMDARs contributes significantly to neuronal loss, while NMDAR dysfunction is thought to contribute to diseases such as Alzheimer's, Parkinson's and Schizophrenia. More recently it is now recognised that de novo mutations in the protein sequence of NMDARs can lead to intellectual disability. Directly related to our structure-function studies of NMDARs we use pre-clinical models of single gene causes of neurodevelopmental disorders (such as fragile X syndrome) to study the properties of altered synaptic function and to assess the extent to which pharmacological intervention can ameliorate the changes that are observed in such models. A more recent focus of our research is the electrophysiological and functional characterization of defined neuronal and glial populations derived from human embryonic stem cells and induced pluripotent stem cells and specifically those from individuals suffering from neurodevelopmental and neurodegenerative diseases. Our work seeks to assess the electrophysiological profile of such neurons in order to further our understanding of these debilitating diseases. Our overall aim is to develop an integrated approach to research that begins with the study of single protein molecules and synaptic function and extends, through collaboration with colleagues, to whole animal studies with an ultimate goal of the clinical study and treatment of disease.

Biographies - Speakers

Slav Bagriantsev - Yale University, USA

Slav Bagriantsev is a Associate Professor of Physiology at Yale University. Our laboratory uses a multi-disciplinary approach to study the molecular basis of sensory physiology, with a focus on mechano- and temperature-sensitivity. We apply electrophysiology, molecular biology and biochemistry to understand the molecular basis of the sense of touch and temperature.

Thomas Baukrowitz - Christian-Albrechts-Universität zu Kiel, Germany

Thomas Baukrowitz is Professor of Physiology at Christian-Albrechts University in Kiel (Germany). The main research interest of my group is to understand the structural mechanisms underlying the complex regulation of K+ channels (such as K2P channel, Kir channel and Kv channels) by membrane voltage, mechanical force, temperature, various lipids and pharmacological compounds.

Sylvie Ducki - Institut de Chimie de Clermont-Ferrand, France

Graduated with a PhD in Organic Chemistry in 1997 from the University of Manchester UK, Sylvie Ducki continued with a postdoc (1998-2000) in Chemistry of Natural Products at the Cancer Research Institute in Arizona State University (USA). Back in Europe in 2000, she worked as a researcher in Medicinal Chemistry at the pharmaceutical company Pharmacia in Nerviano (ITALY). In 2001, she obtained her first academic position as Lecturer in Organic Chemistry at the University of Salford UK, where she was promoted to Senior Lecturer in 2005. In 2007, she joined the National Graduate School of Chemistry of Clermont-Ferrand (ENSCCF which became SIGMA Clermont in 2016) as a university professor in CNU 32, organic chemistry. In Clermont, Prof Ducki is group leader the CESMA team (Design Extraction Synthesis of Analgesic Molecules) within the Institute of Chemistry of Clermont-Ferrand (UMR6296) and Vice-President of the Analgesia Institute.During her career, Professor Sylvie Ducki published over 60 articles in international journals and filed 5 patents. These articles have been cited more than 2700 times (h-index 26).

Fredrik Elinder- Linköping University, Sweden

PhD in Neurophysiology from Karolinska Institutet. Professor of Molecular Neurobiology at Linköping University since 2004. Vice Dean of the Faculty of Medicine and Health Sciences at Linköping University since 2012. My scientific work has focused on the understanding of the mechanisms to open and close voltage-gated ion channels and how to modulate these mechanisms.

Biographies - Speakers

Stefan Feske - New York University, USA

Dr Feske is a Professor in the Department of Pathology at NYU School of Medicine and the Director of the new Ion Channel and Immunity Program at NYU. He graduated summa cum laude with a research thesis and M.D. followed by a residency in rheumatology at the University of Freiburg, Germany. He conducted his postdoctoral studies at the Max-Planck-Institute for Immunobiology and Harvard Medical School where he has made essential contributions to the discovery of the CRAC channel protein ORAI1. Dr Feske identified the first patients with CRAC channelopathy due to mutations ORAI1 and STIM1 genes. Research in his lab at NYU is focused on the functional characterization of ORAI1 and its activator STIM1 and on understanding how CRAC channels regulate immunity to infection, cancer and in autoimmunity. A newer focus of the Feske lab is to systematically investigate which ion channels control immune responses in health and disease with the ultimate goal of identifying new drug targets for immunotherapy.

Marc Freichel - Heidelberg University, Germany

Marc Freichel (Director at the Dept. of Pharmacology, Heidelberg University) is a MD by training and a board-certified consultant in Pharmacology. During his MD thesis (1990/91, Dept. of Medical Biochemistry, Saarland University) he identified regulatory $Ca_V\beta$ subunits of voltage-gated Ca^{2+} channels. During 1995-1997 (Dept. of Internal Medicine and Pharmacology, Heidelberg University) he started his work on the functional role of TRP channels in Veit Flockerzi's lab. In 1998 he moved to the Department of Experimental and Clinical Pharmacology and Toxicology (Saarland University) and identified TRPC4 as an essential constituent of cation channels in endothelial cells. From 2004 to 2011 he was appointed as Associate Professor in Experimental Pharmacology and Preclinical Disease Models and the Head of the Transgenic Mouse Facility at the Medical Faculty. By generating numerous transgenic mouse models with deletions or introduction of defined mutations into genes encoding Ca_V and TRP channels he identified the role for $Ca_V\beta2$ subunits for cardiac development and contraction in the embryonic mouse heart, for TRPM4 as a molecular break for Ca^{2+} entry and activation of mast cells, as well as for catecholamine secretion and blood pressure regulation. With his team, he also discovered TRPV6-mediated Ca^{2+} absorption in the epididymal epithelium as a critical process for the determination of male fertility. He served as a Vice Chairman of the DFG Graduate School 1326 "Calcium signalling and cellular nanodomains". In 2011 he was appointed as Full Professor and Director at the Department of Pharmacology at Heidelberg University. He is a faculty member of the HBIGS Graduate School of Molecular and Cellular Biology, a member of the ethical review committee of the Medical Faculty evaluating clinical drug trials. Since 2012 he is a Principal Investigator in the German Center for Cardiovascular Research (DZHK). Recently, he identified defined heteromeric TRPC channel complexes for hippocampal synaptic transmission and associated memory processes and pathological cardiac remodelling. By applying classical gene targeting and genome editing approaches as well as Adeno-associated viral vectors he aims to identify cation channels and their regulators as drug targets in preclinical models as well as to validate small molecule approaches designed for these targets.

David Hackos - Genentech, USA

David Hackos is a Senior Scientist in the Neuroscience Department at Genentech in South San Francisco. Previously David worked for Roche in Palo Alto and Renovis in South San Francisco and in total has over 15 years of experience in the pharmaceutical industry. His research has focused on targeting ion channels involved in pain sensation with the hopes of developing novel non-opioid pain drugs. David earned a B.A. in physics at Johns Hopkins University and a PhD. in biophysics at UC San Francisco and did postdoctoral research at the NIH in Bethesda MD.

Biographies - Speakers

Sarosh Irani - Oxford Autoimmune Neurology Group, UK

I am a consultant neurologist and clinician-scientist with clinical and laboratory experiences in the field of autoantibody mediated diseases of the nervous system, in particular, the central nervous system. I care for patients with these disorders and run a research group combining clinicians, clinician-scientists and basic scientists to learn more about the origins and treatments of these diseases. The autoantibodies target molecules such as the NMDAR, LGI1, CASPR2 and AMPA/GABARs. My group works to make pathophysiological sense of the phenotypes and the autoantibodies by studying by understanding the mechanisms by which the antibodies are generated and their effects on neuronal models. In particular, we are interested in obtaining immune cells which make autoantibodies from patients and assessing conditions which promote and inhibit antibody production. We anticipate this will provide insights into the mechanisms by which autoantibody production can be inhibited.

Thomas Jentsch - Max-Delbrück-Center for Molecular Medicine, Germany

Thomas Jentsch studied human medicine from 1972 to 1978 at the Free University of Berlin (FU Berlin) and from 1974 to 1980 physics at the FU Berlin. In 1979, he received his medical degree and earned his diploma in physics. Thomas Jentsch graduated in 1982 with a PhD rer. nat. in physics at the Fritz Haber Institute of the Max Planck Society and the FU Berlin and 1984 Dr med. med. at the FU Berlin. Afterwards, he was a research assistant at the Institute of Clinical Physiology at the Charité Berlin on the Campus Benjamin Franklin. Between 1986 and 1988, he was a postdoc in the department of Harvey F. Lodish at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. From 1988 to 1993 Jentsch was Research Group Leader at the Centre for Molecular Neurobiology Hamburg (ZMNH), University Medical Centre Hamburg-Eppendorf. From 1993 to 2006 he was Professor and Director of the Institute of Molecular Neuropathobiology at the ZMNH, from 1995 to 1998 and again from 2001 to 2003 also Director of the ZMNH. Since 2006, Jentsch is full professor at the Charité Berlin. He is head of the research group Physiology and Pathology of Ion Transport at the Leibniz Institute of Molecular Pharmacology and the Max Delbrück Centre for Molecular Medicine. Since 2008 Jentsch is the first researcher of NeuroCure. In 2015, The Journal of Physiology honoured him and his associates with a special issue for the discovery of chloride channels and chloride transporters 25 years ago. On May 2, 2017, the medical faculty of the University of Hamburg awarded him an honorary doctorate.

Fusao Kato - Jikei University School of Medicine, Japan

Graduated from The University of Tokyo in 1982 and received PhD in Pharmaceutical Sciences in 1989 and 2nd PhD in Medical Sciences in 1997. Research associate in Department of Pharmacology, Jikei University School of Medicine in 1984, foreign researcher in l'Institut Alfred Fessard, CNRS, France (1993-1995), Professeur Associé in l'Institut de Chimie Biologique et de la Physiologie, l'Université Louis Pasteur, Strasbourg, France (1995-1996; 1998), and invited researcher in The Institute of Molecular Physiology of University of Sheffield, UK (2001). Full Professor of Jikei University of School of Medicine since 2005. Research interests include synaptic plasticity and glia-neuron interaction at the synapses in the in pain/emotion network including the amygdala and in the central autonomic network.

Sung-Young Kim – Daewoong Pharmaceutical co., Ltd

Sung-Young Kim is a Head of ion channel research team at Daewoong Pharmaceutical co., Ltd at South Korea. Our team focuses on ion channels to develop intractable neurological disease drugs. He did his PhD at the physiology department at the Seoul National University studying the TRP channel (Advisor: Insuk So). After this, he joined Daewoong Pharma and is conducting research related ion channel drug development.

Biographies - Speakers

Dimitri Kullmann - UCL Institute of Neurology, UK

Dimitri Kullmann is a professor of neurology at the UCL Queen Square Institute of Neurology, London. He trained in medicine and physiological sciences in Oxford and London and holds a DPhil from Oxford. After a post-doctoral fellowship at UCSF with Roger Nicoll, he completed neurology training in London. His laboratory has contributed to the discovery of 'silent synapses', extrasynaptic actions of glutamate and GABA in the brain, long-term potentiation in different subtypes of interneurons, and mechanisms of neurological channelopathies. His current interests include mechanisms and computational roles of gamma oscillations and gene therapy for epilepsy. He is a Fellow of the Royal Society and of the Academy of Medical Sciences and is the Editor of Brain.

Yoshihiro Kubo - National Institute for Physiological Sciences (NIPS), Japan

Yoshihiro Kubo is Professor and Chief Chairperson of National Institute for Physiological Sciences (NIPS), Japan. He graduated from University of Tokyo, Faculty of Medicine in 1985, and completed a PhD course in Medical Sciences, University of Tokyo in 1989. He served as Chief Research Scientist in Tokyo Metropolitan Institute for Neuroscience from 1989 to 2000. During this period, he joined Prof Lily Jan's lab in the University of California, San Francisco from 1991 to 1993 as a Post-doc, where he succeeded in the cDNA cloning of IRK1(Kir2.1) and GIRK1(Kir3.1) channels. He was recruited as a Professor of Tokyo Medical and Dental University, Graduate School of Medicine in 2000, and moved to NIPS as a Professor in 2003. He served for The Journal of Physiology for 14 years, as an Editor (2004-2018), Senior Editor (2010-2018) and Deputy Editor-in-Chief (2011-2016). His research interest is the functioning mechanisms of ion channels and receptors, and focuses on their structure-function relationships and dynamic structural rearrangements, using electrophysiological and opto- physiological approaches. His lab worked so far on the inward rectifier K+ channels (IRK1, GIRK1), voltage-gated K+ channel (KCNQ1/ KCNE1 complex, hERG, Kv4.2/ KChIP4/ DPP10 complex), Two Pore Na+ channel (TPC3), ATP receptor channel (P2X2), and G-protein coupled receptors (mGluR1, GABABR, muscarinic receptors).

Jack Mellor - University of Bristol, UK

Jack Mellor graduated from the University of Cambridge in 1995 with a degree in Neurophysiology and stayed in Cambridge to study for his PhD on the biophysics and pharmacology of inhibitory synaptic transmission at the MRC Laboratory of Molecular Biology with Andrew Randall. After completing his graduate work in 1998, Jack worked briefly on science policy at the UK government before joining Roger Nicoll's laboratory at the University of California San Francisco working on the mechanisms of synaptic plasticity in the hippocampus. In early 2002 Jack returned to the UK and joined the laboratory of John Isaac at the University of Bristol before setting up his own laboratory in 2004 with an MRC funded Career Development Fellowship. He now leads a team that focusses on how hippocampal network function is modified by synaptic plasticity and neuromodulators.

Paul Miller - Cambridge University, UK

Paul Miller has recently joined University of Cambridge as a new lecturer in the Pharmacology Department. Previously he spent 8 years as a postdoc in the Division of Structural Biology at University of Oxford where he used structural biology techniques to solve structures of GABA-A receptors. Before this he studied and did a postdoc at University College London, using electrophysiology and pharmacological approaches to link structure and function for, principally, Glycine receptors. Currently, Paul combines structural and functional approaches to better understand how GABAA receptors are modulated by ligands. This includes using structural data to guide the design of novel small molecule ligands. Most recently, Paul has contributed to the study of over 100 unique nanobodies (Nb; miniature antibodies), some of which are positive allosteric modulators (PAMs) of mouse/rat/human GABAA receptors. These are being studied as useful tools to dissect neuronal functions performed by distinct subtypes, and investigated for therapeutic potential. A main theme in the lab is to study, engineer and produce membrane proteins for use directly as pharmacological tools, and to guide the design of ligands against them, both of which will help to better understand neurological systems and inform on therapeutic development.

Biographies - Speakers

Isabel Pérez-Otaño - University of Alicante, Spain

Dr Pérez-Otaño received her Ph.D. from the University of Navarra, Spain where she developed an interest in neuroscience and brain disease. She took postdoctoral training with Professor Steve Heinemann at the Salk Institute for Biological Studies in San Diego and later worked with Drs Michael Ehlers and Don Lo at the Neurobiology Department of Duke University on the cell biology of glutamatergic neurotransmission. In 2004, she joined the Neuroscience Department of the Center for Applied Medical Research of the University of Navarra Medical School, which she directed from 2014 until accepting her current post. Her lab aims to identify key cell biological pathways that guide the development and refinement of synaptic circuits and explores links of these pathways to mood, cognition and brain disease. Over the last decade, her work has focused on the properties and roles of a new class of glutamate-type NMDA receptors containing GluN3A subunits (GluN3A-NMDARs) in neural circuit development and animal behaviour. She is a recipient of Young and Independent Investigator Awards from the National Alliance for Research in Schizophrenia and Depression and has been awarded Research Grants from the Hereditary Disease Foundation (USA), Marie Curie Program and Spanish Government funding agencies. She has been a Visiting Scholar at Stanford University (2010) and participates as an independent expert advising the European Commission and UK Welcome Trust on science funding. Recent discoveries from her group described aberrant reactivation of synaptic pruning as an early cause of Huntington´s disease, and her team is undertaking a translational effort to translate this knowledge into therapies.

Guillaume Sandoz - University of Côte d'Azur, France

Guillaume Sandoz (Research Director, CNRS) is a molecular and cellular neuroscientist with a strong background in ion channel physiology and pathophysiology. During his PhD (2000-2004), he studied the regulation of pre-synaptic voltage-dependent calcium channels. After his doctoral studies, he joined the Lazdunsky and Lesage labs where he developed a proteomic approach to identify proteins which interact with two-P-domain potassium (K2P) channels. In 2009, he joined the Isacoff lab at UC Berkeley as a Fulbright Visiting Scholar. With this group, he discovered a new family of ion channels (AvGluR) and developed a new method (photo-conditional subunit) to endow light sensitivity to endogenous channels. Using this tool, he found that K2P2.1, typically considered to be a leak channel, contributes significantly to the hippocampal GABAB which is involved in sIPSP. In 2012, Guillaume obtained the French ATIP-AVENIR grant, allowing him to start the "Biology of Ion Channels" lab at the iBV, in Nice, France. His group works on elucidating the physiological and pathological implications of K2P channels using optogenetic and single molecule techniques. Previous work from his group has elucidated a link between alcohol-induced neuronal excitability and K2P channel inhibition. More recently, his team has discovered a novel mechanism which causes migraines, opening a new path for the development of medication for treating migraines.

Mala Shah - UCL School of Pharmacy, UK

Mala Shah completed her PhD in the Department of Pharmacology, University College London (UCL) during which she worked on understanding the pharmacology of the slow afterhyperpolarization current, resulting in the identification of a novel inhibitor, UCL 2077, of the current. She subsequently obtained a Wellcome International Prize Travel Fellowship to work with Prof. D. Johnston at Baylor College of Medicine (USA). During this time, she discovered that the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels were persistently reduced following seizures, leading to cortical neuronal hyperexcitability. In 2004, she returned to UCL as a senior fellow and showed that the subthreshold-active K+, KV7, channels, contributed to maintaining the action potential threshold in axons. She obtained her lectureship at School of Pharmacy in 2007. Her lab's interests include understanding the function and modulation of cortical and hippocampal neuronal ion channels under physiological and pathophysiological conditions.

Biographies - Speakers

Insuk So – Seoul National University, Korea

Insuk So graduated at the College of Medicine, Seoul National University in South Korea and then received his PhD in Physiology at the same college. He finished postdoctoral training with Peter Stanfield at Leicester University. In 1994, he established an independent smooth muscle research group at the Department of Physiology, College of Medicine, Seoul National University right after his employment as a full university professorship. He studied the role of muscarinic acetylcholine receptor-activated nonselective cation channels in smooth muscle cells and showed that Transient Receptor Potential Canonical (TRPC) 4 is the molecular candidate. Since then, for nearly 25 years, his research has been mainly focused on molecular physiology of TRP cation channels (TRPM, TRPC and TRPV subfamilies mediated different pathways of calcium-dependent signal transduction). Recently, he is very interested in studying TRP channels, particularly TRPC channels, in the context of protein-protein interaction by resolving the complex relationship between TRPC channels and G proteins. He considers direct interaction between TRPC channel proteins and G proteins as the most important clue for understanding this issue.

Alexander I. Sobolevsky - Columbia University

Alexander Sobolevsky (Columbia University) earned his PhD in biophysics in 1999 from the Moscow Institute of Physics and Technology, working under guidance of Prof. Boris Khodorov. He held his first postdoctoral position in 2000, studying physiology and neuroscience with Dr Lonnie Wollmuth at the State University of New York, Stony Brook. He continued his training in biochemistry and biophysics with Dr Eric Gouaux, first at Columbia University and then at the Oregon Health and Science University. He joined Columbia University's faculty in 2010 as an Assistant Professor of biochemistry and molecular biophysics, focusing on structure and function of ion channels. In 2017, he was promoted to an Associate Professor.

Thomas Voets - University of Leuven

Thomas Voets graduated as a chemical engineer in 1993, obtained a postgraduate degree in cellular biology in 1994, and a PhD in Biomedical Sciences in 1998 under the guidance of Prof. Bernd Nilius, all at the University of Leuven. From 1998 to 2001, he performed postdoctoral research at the Max-Planck Institute for Biophysical Chemistry in Göttingen (Germany), in the laboratory of Professor Erwin Neher. In 2002, he was appointed Assistant Professor at the University of Leuven, Faculty of Medicine, where he teaches Cell Biology, Cell Physiology and Biophysics to (bio)medical students. Since 2010, he is full Professor and chairman of the Laboratory of Ion Channel Research within the Department of Cellular and Molecular Medicine at the University of Leuven. Since 2017, he combines this professorship with a group leader position within the VIB-KU Leuven Center for Brain and Disease Research.
Thomas Voets published more than 200 papers in international biomedical research journals. His recent research focuses on Transient Receptor Potential (TRP) ion channels, molecular gateways for ions in the membranes that surround the cells in our body. The opening and closing of these TRP channels initiate calcium signals and electrical impulses that underlie key processes in various cells and tissues, including the central and peripheral nervous system, the heart, the musculoskeletal system and kidneys. Dysregulation of TRP channel function is the cause of various severe inherited and acquired human diseases. The central aim of the research team of Thomas Voets is to provide better insight into the aetiology of TRP-related diseases and to use this knowledge to develop novel therapeutic strategies for patients. In particular, recent research by his team revealed the fundamental roles of TRP channels in acute and chronic pain, and form the basis of translational research aimed at developing novel TRP channel-based analgesic drugs.

Paul Wright - LifeArc

Paul Wright is a Principal Scientist at the LifeArc Centre of Therapeutics Discovery, based in Stevenage, UK. Paul specializes in the development of cell-based assays to study ion channels and GPCRs and has a particular interest in using new screening technologies and quantitative pharmacology to identify ligands for novel or previously undrugged targets. Most recently this work has focused on identifying and developing molecules to help advance potential therapeutics targeting two-pore domain potassium (K2P) channels. Paul is also part of the LifeArc Neuroscience disease area leadership team. Paul completed a PhD in Neuroscience at the Institute of Psychiatry, King's College London and post-doctoral training at the University of Massachusetts Medical school and Harvard Medical School.

Poster Abstracts

Biophysical and pharmacological profiling of multiple voltage-gated sodium channel subtypes on QPatch II

Mads P G Korsgaard
Sophion Bioscience

Poster board 01

Voltage-gated sodium channels (VGSC) are responsible for the initiation and propagation of action potentials in excitable cells. VGSC have been identified as excellent drug targets for treatment of pain, epilepsy and to other neurological disorders. Early compounds, however, were developed using empirical approaches. The identification of the molecular identity of VGSC in combination with technological advances, such as the automated patch clamp technique, provide the basis for a rational design of subtype-selective compounds.
To date, 9 functional mammalian isoforms ($Na_V1.1$–1.9) have been described in the literature. The various subtypes differ in their expression pattern and exhibit distinct biophysical and pharmacological profiles. All have in common that they produce a transient inward current in response to membrane depolarization. During this process the VGSC transitions from a closed to and open into an inactivated state. Interestingly, inhibitor compounds often exhibit different pharmacological profiles dependent upon the ion channel conformational state.
In the present study, the second generation QPatch (QPatch II; Sophion Bioscience) was used in combination with adaptive voltage protocols to investigate state-dependent inhibition of tetrodotoxin (TTX) and tetracaine on 8 different VGSC subtypes ($Na_V1.1$-8). A first step was to determine the half inactivation potential V½(inactivation) for each individual cell. This value was then used during the next steps as preconditioning pulse. Such an adaptive protocol allowed to determine IC_{50} values for both the closed and the inactivated state and reduce heterogeneity of the cells. Both IC_{50} values and biophysical parameters of the different subtypes align well with literature values.

iPSC-derived motor neurons on the automated patch clamp platforms Qube and QPatch

Melanie Schupp
Sophion Bioscience

Poster board 02

Human induced pluripotent stem cells (hiPSCs) can be differentiated into multiple cell types, including neurons and cardiomyocytes. This gives rise to a novel way of establishing human disease models, which in turn can be used for drug development in vitro. Ion channels represent highly attractive therapeutic targets in the nervous and the cardiovascular system, rendering electrophysiological studies of hiPSCs important for their usage in drug discovery. However, such studies have traditionally been limited by the labor-intensive and low-throughput nature of patch-clamp electrophysiology. Here we use our automated patch clamp systems Qube 384 and QPatch 48 in order to increase throughput and reduce time lines.
Our observations include channel expression versus time in culture, the pharmacological dissection of endogenous ion channels (e.g. Na_V and K_V), identification of ligand-gated receptors, and recordings of action potentials using the current clamp feature. Also, we show the electrophysiology of a spinal muscular atrophy (SMA) and an amyotrophic lateral sclerosis (ALS) model. The disease model for SMA was derived by mutations in the SMN1 gene and shows enhanced sodium channel activity but no shift in the normalized current voltage relationship. ALS was here mimicked by a single point mutation in the superoxide dismutase 1 protein (SOD1), D90A, which had previously been identified in recessive, dominant and seemingly sporadic pedigrees. Cells carrying this point mutation displayed larger sodium currents, which eventually led to neurofilament aggregation, neurite degeneration and other phenotypes. We could confirm that the electrophysiological effect could be reversed by point mutation to D90D.
Our measurements validate the feasibility of measuring hiPSC ion channel currents using the APC platforms Qube and QPatch. Altogether, these results can facilitate evaluating the use of hiPSC for early drug development and in extension personal medicine.

Exploring the benefits of slow inhibition for visual processing in the thalamus

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Poster board 03

In the visual thalamus, slow-rising inhibitory postsynaptic conductance's (IPSCs) are believed to be a feature of synaptic inhibition generated by local interneurons of the visual thalamus. Genetic deletion of these local interneurons results in the absence of these slow-rising IPSCs and optogenetic experiments further demonstrated the delayed nature of dendro-dendritic GABA release. Dynamic-clamp experiments demonstrated the complementary features of fast and slow inhibition for the control of relay neuron excitability both in terms of gain change and information content. We demonstrate that delayed feedforward inhibition is an effective alternative to feedback inhibition arising from the surrounding reticular nucleus. Finally, we discuss the energy saving benefits of providing inhibition through local interneurons and propose that increased neuronal complexity drives the requirement for inhibition from local interneurons.

Poster Abstracts

Optical modulation of ion channels using Qube Opto

Anders Lindqvist
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Poster board 04

The discovery of light-activated ion channels has paved the way for many exciting developments in the field of optogenetics. These ion channels change their conformation following optical stimulation allowing ions to pass through their pore. Since their discovery, many genetically engineered versions have been generated, exhibiting a broad spectrum of biophysical properties. Light can further be used to indirectly modulate ion channels through the use of caged or photoswitchable compounds, or by optically activating secondary messenger pathways. Optical modulation of ion channels is traditionally studied using a manual patch clamp system combined with a light source. This approach, however, is limited by a very low throughput. In the present work we present data recorded using a 384-well based automated patch clamp system equipped with 384 build-in light sources (Qube Opto).

A pharmacological synopsis of small molecules, toxins and CiPA compounds targeting human cardiac $K_V4.3$ channels

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Sanofi-Aventis

Poster board 05

Abstract: $K_V4.3$ α-subunits associate with ancillary α-subunits of the KChIP2 family in heart muscular tissue to channel transient outward (I_{to}) currents. Decreased expression or dysfunction of $K_V4.3$ channels following myocardial infarction or during heart failure can contribute to abnormal repolarization, which may result in ventricular arrhythmias. Therefore, drug-induced inhibition of $K_V4.3$/KChIP2-mediated Ito exposes to potential cardio safety liabilities which are important to document early during the drug discovery process. In this study we have characterized basic electrophysiological properties pertaining to Ito currents in CHO cells expressing recombinant $K_V4.3$ and KChIP2.2 subunits. The currents obtained were validated pharmacologically by assessing the efficacy of small molecule and peptide toxin inhibitors known from manual patch-clamp studies to block Ito. We then examined the inhibitory activities of 28 drugs with clinically documented high, medium or low pro-arrhythmic risk encompassing the test- and validation-sets of the Comprehensive in vitro Pro-Arrythmia (CiPA) panel. While none of the CiPA drugs inhibited $K_V4.3$ at submicromolar concentrations (IC_{50} < 1 μM), two drugs (loratadine and nitrendipine) displayed intermediate potency (1 μM < IC_{50} < 10 μM), and four drugs (bepridil, quinidine, chlorpromazine and astemizole) revealed weak, but significant (10 μM < IC_{50} < 30 μM) inhibitors.

In silico identification and electrophysiological characterisation of novel acid-sensing ion channel 3 (ASIC3) modulators

Gerard Callejo
University of Cambridge

Poster board 06

Acid-sensing ion channels (ASICs) are voltage-independent cation channels activated by extracellular protons. They are involved in pain, fear, learning, and neurodegeneration after ischemic stroke. Of all the ASIC subunits, ASIC3 has been suggested as the key sensor of acid-induced pain and has also been shown to have a pivotal role in different models of inflammatory pain including models of rheumatoid arthritis and osteoarthritis. Therefore, the identification of new ASIC3 modulators and the mechanistic understanding of how these modulators modulate ASIC3 could be important for the development of new strategies to counteract the detrimental effects of dysregulated ASIC3 activity in inflammatory conditions. Here, we report the identification of novel ASIC3 modulators based on the ASIC3 specific agonist, 2-guanidine-4-methylquinazoline (GMQ) and the ASIC3 specific inhibitory toxin APETx2. Through an *in silico* ligand (here, GMQ)-guided screening of FDA-approved drugs, we selected 5 different compounds and tested them against ASIC3 using whole-cell recording. Of the chosen drugs, guanabenz, an α2-adrenoceptor selective agonist, produced similar effects to GMQ on ASIC3, activating the channel at neutral pH and potentiating its response to mild acidic stimuli. Sephin1 is a guanabenz derivative that lacks α2-adrenoceptor activity and has been proposed to act as a selective inhibitor of a regulatory subunit of the stress-induced protein phosphatase 1 (PPP1R15A). However, we found that like guanabenz, sephin1 activates ASIC3 at neutral pH and potentiates its response to acidic stimulation, i.e. sephin1 is a novel modulator of ASIC3. Besides this ligand-guided approach, we also modelled the interaction of the selective peptide inhibitor of ASIC3 APETx2 and predicted two regions of the APETx2 sequence that mediate interaction with ASIC3. Based on this, we designed peptides predicted to bind to the ASIC3-APETx2 binding sites and identified a peptide that inhibits the transient component of ASIC3 current whilst also dramatically increasing its inactivation time constant; a control peptide where proposed ASIC3-binding residues have been mutated to alanine had no such effect. Overall, these data demonstrate the utility of computational analysis for identifying novel ASIC3 modulators, which can be validated with electrophysiological analysis and may lead to the development of better compounds for targeting ASIC3 in the treatment of inflammatory conditions.

Poster Abstracts

$K_V3.1$ and $K_V3.3$ differentially contribute to action potential repolarization in principal neurons of the auditory brainstem

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Poster board 07

The 'delayed rectifier' potassium current is mediated by K_V3 channels which open at voltages during action potential (AP)-mediated depolarisation and thus provide the repolarization drive to support high-frequency firing in neurons. Multiple K_V3 genes are co-expressed in several parts of the brain, either as hetero- or homo-tetramers but subunit specific roles are largely unknown. Neurons of the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO), in the superior olivary complex (SOC) of the auditory brainstem, compute sound source localisation through integration of binaural stimuli. Both MNTB and LSO neurons express $K_V3.1$ and $K_V3.3$ subunits. We have used this neuronal network to investigate role of these subunits in generating functional KV3 channels and during repolarization of the postsynaptic AP. The study was conducted on CBA/Ca mice, and on $K_V3.1$ knockout (KO), and $K_V3.3$KO mice backcrossed onto the same strain. Expression of K_V3 channels in the MNTB and LSO principal neurons was investigated by qRTPCR, western blot and immunohistochemistry. For *ex vivo* whole cell patch-clamp electrophysiology, transverse sections of SOC were used for recording currents and voltages in the neurons under different biophysical and pharmacological conditions. Intracellular Ca^{2+} transients were studied by Fura-2 ratiometric fluorescence measurement. Statistical significance was determined using Student's t-test and one-/two-way ANOVA and expressed as mean ± SD.

The results obtained reveal that MNTB neurons effectively employed either $K_V3.1$ or $K_V3.3$ subunits in K_V3 channels, with similar whole-cell current amplitude; however, the fastest APs were achieved when both subunits were expressed together (AP halfwidth-WT: 0.29 ± 0.07ms, n=14; $K_V3.1$KO: 0.41 ± 0.11ms, n=11, p=0.0207; Kv3.3KO: 0.48 ± 0.06ms, n=11, p<0.0001). In the LSO, $K_V3.3$ subunits were essential; $K_V3.1$ mRNA was present, but somatic K_V3 channels must contain at least one $K_V3.3$ subunit, since $K_V3.3$KO mice had little or no TEA-sensitive K_V3 current (Kv3.3KO: 6.0 ± 2.5nA, n=11; with TEA: 5.0 ± 1.8nA, n=7), and AP halfwidths were increased compared to WT (WT: 0.28 ± 0.04ms, n=10; $K_V3.3$KO: 0.69 ± 0.15ms, n=7, p<0.0001). Measurement of whole-cell voltage-gated Ba^{2+} currents showed no change in the MNTB or LSO, but the longer AP duration in the KOs increased $[Ca^{2+}]i$ in an activity-dependent manner. We conclude that K_V3 channels make major contributions to AP repolarization in both the MNTB and LSO. $K_V3.1$ and $K_V3.3$ subunits each contribute to K_V3 currents in the MNTB but $K_V3.3$ is crucially dominant for K_V3 channels in the LSO, while both of the subunits influence Ca^{2+} influx in these neurons.

Stellate ganglia ion channel dysfunction leads to repetitive firing in the spontaneously hypertensive rat

Harvey Davis
University of Oxford

Poster board 08

Sympathetic hyperactivity is a hallmark of hypertension, but its molecular mechanisms are unknown. A key aspect of neuronal information encoding is firing rate, however this fundamental property is unstudied in the cardiac innervating sympathetic stellate ganglia. We hypothesised that stellate ganglia neurons would have higher firing rates in the spontaneously hypertensive rat (SHR) than age-matched normotensive Wistar controls. Using RT-qPCR, single-cell RNA-seq and immunohistochemistry, we observed expression of M-current subunits in stellate ganglia, with decreased transcript expression in the SHR. We then confirmed M-current transcript expression in human tissue by RT-qPCR. Through perforated patch recordings of stellate ganglia neurons from Wistar and SHRs, we observed that SHR neurons exhibited tonic firing compared to the phasic firing of Wistar neurons. M-current deactivation curves revealed a decrease in SHR M-current. Further to this, M-current inhibition with Linopirdine or XE991 induced tonic firing in Wistar neurons. Conversely, the M-current activator retigabine reduced firing rate in SHR neurons. This state appeared to be dependent upon an additional channel, as not all SHR neurons fired repetitively even after M-current inhibition. Indeed, our data indicate that $Na_V1.8$ inhibition prevents repetitive firing in SHR neurons and in M-current inhibitor treated Wistar neurons. This phenomenon was not observed in other channels associated with neuronal firing rate. These data support the hypothesis that decreased M-current in the SHR stellate ganglia leads to a phenotype of increased neuronal firing and may represent a key component of sympathetic activity associated with hypertension.

Poster Abstracts

New CiPA cardiac ion channel cell lines and assays for in vitro proarrhythmia risk assessment

Edward Humphries
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Poster board 09

New cardiac safety testing guidelines are being developed as part of the FDA's Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, which aims to remove the reliance on screening against the hERG channel by expanding the panel to include $Na_V1.5$, $Ca_V1.2$, $K_V4.3$/KChiP2.2, $K_{ir}2.1$ and $K_V7.1$/KCNE1. In addition, the CiPA working groups have recently identified two additional in vitro assays required for in silico models to reliably predict proarrhythmia. The first is a late sodium current assay, as inhibition of persistent inward current can affect repolarisation and mitigate proarrhythmia (e.g. Ranolazine). The second assay determines the kinetics of drug trapping in the hERG channel using the Milnes voltage protocol (Milnes et al, 2010), which can improve the prediction of proarrhythmia risk (Li *et al.* 2018). Here we have validated these additional assays on the gigaseal quality QPatch 48 automated patch clamp platform. Rather than rely on pharmacological activators such as ATX-II or Veratridine to induce late openings of the $Na_V1.5$ channel, we created a HEK cell line expressing the LQT3 ΔKPQ $hNa_V1.5$ mutant which promotes activation of persistent sodium currents (Chandra, Starmer & Grant, 1998). Late sodium current was measured using step pulse, step-ramp, ramp, and action potential waveform voltage protocols in proprietary solutions to minimise rundown and increase whole-cell seal duration. ΔKPQ $hNa_V1.5$ cells exhibited Ranolazine-sensitive late sustained inward current with an ~1-3% magnitude of the peak current (>4 nA). A CHO cell line expressing the hERG channel and QPatch whole-cell conditions were applied to published Milnes hERG kinetic protocol to create a biophysically and pharmacologically validated QPatch assay. We compensated for slower activation kinetics at room temperature (compared to manual patch at 37°C) and optimised liquid addition and voltage protocols to create a stable assay during prolonged vehicle applications. Our QPatch Milnes hERG assay correctly reported the drug trapping profiles of cisapride (10%) and dofetilide (95%).
In conclusion, we developed a late $Na_V1.5$ ΔKPQ mutant cell line and a Milnes hERG kinetic assay on a gigaseal quality screening platform that meet the additional requirements of the CiPA initiative, and can provide high quality HTS data for more accurate cardiac safety assessment.

Generating potent and selective inhibitors of $K_V1.3$ ion channels by fusing venom derived mini proteins into peripheral CDR loops of antibodies

Lien Moreels
IONTAS Ltd.

Poster board 10

Pathogenic T cell effector memory (TEM) cells drive many autoimmune disorders and are uniquely dependent on the $K_V1.3$ channel. A number of venom derived knottin (cysteine-rich mini-protein) inhibitors of $K_V1.3$ are being developed as potential drug candidates, but can suffer from manufacturing difficulties, short half-lives and a lack of specificity. We have developed a novel molecular format wherein a peripheral CDR loop of an antibody has been replaced by a knottin. In this novel KnotBody™ format, the knottin benefits from the improved therapeutic functionality of an antibody and the antibody gains additional diversity by the addition of a scaffold which is pre-disposed to blockade of ion channels.

A proof-of-concept fusion protein of one structural domain within another was initially achieved by inserting a trypsin inhibiting knottin (EETI-II) flanked by diverse repertoire of short linker sequences into the CDR2 position of naïve antibody light chain sequences. Functional KnotBody™ molecules were selected from this library using phage display technology on the basis of retained trypsin binding, with the correct folding of both domains confirmed using X-ray crystallography.

To further demonstrate the benefits of this novel format, the modular nature of the KnotBody™ binding surface was exploited to: (i) improve existing knottin binding by introducing additional VH contacts; (ii) create a bispecific molecule by introducing a VH chain that binds to a different target; (iii) substitute the proof-of-concept knottin (EETI-II, a trypsin inhibitor) with ShK, a $K_V1.3$ ion channel blocking toxin; (iv) develop a panel of low-nM $K_V1.3$ inhibitors with selectivity exceeding 3000-fold over the $K_V1.1$ channel, a closely related Kv family member.

Poster Abstracts

Spadin selectively antagonises arachidonic acid activation of TREK-1 channels

Anthony Lewis
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Poster board 11

We recently revealed TREK-1 to be a critical regulator of murine intestinal contractility using a variety of TREK-1 specific activators (Ma *et al*, 2018). Interestingly, a proposed TREK-1 specific inhibitor, spadin, was unable to perturb agonist-induced changes to smooth muscle contractility. Since the mechanism of action of spadin was unclear, we hypothesised that it may possibly regulate TREK-1 activity via an activator-specific mechanism. Using heterologous expression in *Xenopus laevis* oocytes and electrophysiological analysis using two-electrode voltage clamp, we characterised the pharmacological profile of wild type and chimeric murine TREK-1 and TREK-2 channels using previously established K2P activators (AA, DHA, BL-1249 and CDC) and inhibitors (spadin and Ba^{2+}). Mouse (m) TREK-1 and TREK-2 channel currents were both significantly increased by AA, BL-1249 and CDC, similar to their human homologues. Under basal conditions, both mTREK-1 and mTREK-2 currents were insensitive to application of spadin, but could be blocked voltage-dependently by Ba^{2+}. Furthermore, spadin did not significantly inhibit either mTREK-1 or mTREK-2 currents following pre-activation by either AA, BL-1249 or CDC. However, pre-exposure to spadin significantly perturbed the activation of mTREK-1 currents by AA, but not BL-1249, CDC or DHA. In mTREK-2 channels, pre-exposure to spadin did not antagonise current activation by either AA, BL-1249 or CDC. The site of AA activation in TREK-1 channels is proposed to be the proximal c-terminus, therefore we hypothesised spadin may interfere with AA binding at this site. Generation of a mTREK-2/mTREK-1 chimera revealed the c-terminus of TREK-1 is not the site of spadin action. These data demonstrate spadin specifically antagonises the activation of TREK-1 channels by AA via an allosteric mechanism.

References

Ma *et al.* (2018) *Front Physiol* ***9***; 157. doi: 10.3389/fphys.2018.00157.

Structure-based identification of novel KNa1.1 inhibitors

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Poster board 12

Several types of drug-resistant epilepsies of infancy have been associated with mutations in the KCNT1 gene, which encodes the sodium-activated potassium channel subunit KNa1.1. Approximately forty different amino acid substitutions have been identified in patients, involving both transmembrane and intracellular domains of the channel subunit, and with one exception all cause gain-of-function in channel gating. Channel inhibition, therefore, is potentially a stratified approach to treat the disorder. To date, quinidine therapy has been trialled with several patients, but mostly with unsuccessful outcomes, which have been linked to its low potency and lack of specificity; effects on cardiac ion channels being a major concern. Here we describe the use of high-resolution structures of KNa1.1 channels, generated through cryo-electron microscopy, to identify novel inhibitors using computational methods. From an initial analysis of seventeen compounds identified using this approach we found six that inhibited KNa1.1 channels. Their IC_{50} values ranged between 0.6 and 7.4 μM, compared to 100 μM with quinidine, and likely inhibited through pore-blocking mechanisms. The compounds delivered varying results when evaluating state-dependence and in preliminary toxicity assays, with some demonstrating negligible cellular toxicity and low levels of hERG block. These compounds may provide starting points for the development of novel pharmacophores for KNa1.1 inhibitors, with the view to treating KCNT1-associated epilepsies. Furthermore, this illustrate the potential for utilising ion channel structures generated through cryo-electron microscopy in drug discovery.

Poster Abstracts

Development of a novel screening system to identify activators of Two-pore domain potassium channels (K2Ps)

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LifeArc

Poster board 13

Two-pore domain potassium channels (K2Ps) are characterised by their four transmembrane domain, two-pore topology. They carry background (or leak) potassium current in a variety of cell types, including those with important pathophysiological roles. However, they have proved a difficult target class to modulate with small molecules and there is a lack of useful specific pharmacological tools which target K2Ps. This in turn has limited the interrogation of the precise physiological function of K2Ps and efforts to generate K2P targeting therapeutics.

The aim of this work was to develop a cellular system to identify activators across all the described subclasses of K2P channels. Generation of cell lines stably over-expressing ion channels can be challenging for a number of reasons including inherent toxicity. Moreover, the ability to identify channel activators can be compromised by systems in which the target is expressed at high levels. To avoid these issues we used BacMam to express Ion channels in mammalian cells. BacMam offers a number of advantages, including safety and reduced time, compared to generating stable cell lines, but importantly it allows the precise titration of expression of the gene of interest. This enabled us to generate cell systems in which we were able to intricately and robustly select a level of K2P expression in functional assays, optimized for the identification of channel activators.

Spontaneously opening gabaa receptors decrease excitability and tune coincidence detection in hippocampal granule cells

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Poster board 14

Background
Neurons express a wide variety of neurotransmitter receptors which allow them to communicate with each other. Activation of these receptors causes either excitation or inhibition in the neuron. The main inhibitory receptor – and the topic of my PhD – is the $GABA_A$ receptor. The classical view of $GABA_A$ receptors signalling in the brain is simple: they are active in the presence of GABA, and – just as importantly – they fall silent in its absence. However, two studies (McCartney, 2007 and Wlodarczyk AI, 2013) demonstrated that this was a gross oversimplification. They showed that in the hippocampus $GABA_A$ receptors exhibit constitutive activity, meaning that they are spontaneously active even in the absence of GABA.

Rationale
When I started my PhD, although the presence of spontaneously active $GABA_A$ receptors had been confirmed, their function remained elusive. During my PhD I was able to address this ambiguity by performing patch-clamp recordings from the rat dentate gyrus.

Results and Conclusions:
1. Spontaneously opening $GABA_A$ receptors produce tonic inhibitory currents in the dentate gyrus. The inhibitory charge delivered by spontaneous $GABA_A$ receptors is 20-fold greater than that produced in the presence of GABA (phasic and tonic inhibition combined). Thus, they cannot be discounted as a mere artefact.
2. These spontaneous receptors are outwardly rectifying, meaning that they deliver more inhibition at depolarised membrane potentials. When the receptors are blocked the passive and active membrane properties of the neurons change so that they become hyper-excitable (higher input resistance, lower rheobase).
3. The entire function of the dentate gyrus network is altered as a result: neurons are no longer able to filter incoming synaptic signals as effectively – in a coincidence detection experimental paradigm neurons fired action potentials when previously they had been silent. However, LTP amplitude was not altered.
4. Finally, I probed the pharmacology of the receptors and found that Midazolam (potent anti-convulsant) and Zolpidem (anxiolytic) both increase the amount spontaneous receptor activity.

Poster Abstracts

Resin acid derivatives open the $hK_V7.2/7.3$ channel and prevent epileptic activity in zebrafish larvae

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Poster board 15

The voltage-gated potassium channel $hK_V7.2/7.3$ has important functions in setting the resting membrane potential and thereby in regulating excitability of neurons. It is a target for the antiepileptic, channel-opening drug retigabine, which has caused adverse effects and was withdrawn from the market. A major adverse effect of retigabine was its channel-opening effects on the $hK_V7.4$ channel. However, retigabine validated opening of the $hK_V7.2/7.3$ channel as an antiepileptic strategy. The aim of this project was to develop $hK_V7.2/7.3$ channel openers with improved ion channel selectivity. To this end, we developed derivatives of naturally occurring resin acids found in resin from pine trees and tested the effect of these compounds on different voltage-gated ion channels. Human K_V7 channels were expressed in oocytes from the Xenopus laevis frog and currents were measured using the two-electrode voltage-clamp technique whilst $hK_V11.1$ and $hNa_V1.5$ were expressed in cell lines (CHO and HEK-cells respectively) and measured using automated patch clamp (QPatch) in collaboration with Sophion Bioscience A/S. We found that specific generated resin acid derivatives were as potent $hK_V7.2/7.3$ channel openers as retigabine. Several of our resin acid derivatives had almost no effect on hKv7.4 and very small structural alterations of the compounds largely altered the effects on $hK_V7.4$. To further investigate potential off-target effects we tested our compounds on the cardiac channels $hK_V7.1$ co-expressed with KCNE1, and on $hK_V11.1$ (hERG). Block of these channels can cause cardiac arrhythmia and subsequent death. None of our investigated compounds blocked any of these channels at 10 µM (estimated highest concentration for clinical effects) and they neither had an effect on the cardiac $hNa_V1.5$ channel. To investigate the antiepileptic effect of our compounds we induced epileptic seizures in zebrafish larvae by the GABA-A receptor antagonist pentylenetetrazol (PTZ) and recorded the electrical brain activity with extracellular electrodes. The epileptic seizures were prevented by retigabine (at 10 µM) and our potential lead compounds had similar effects at the same concentration. By opening the $hK_V7.2/7.3$ channel and preventing epileptic seizures in an in vivo model, our resin acid derivatives thus show the potential to develop into a new type of antiepileptic drug.

Role of VRAC in insulin secretion

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Poster board 16

The Volume-Regulated Anion Channel (VRAC), a key player in regulatory volume decrease, is constituted by LRRC8 heteromers (1), containing the obligatory LRRC8A protein (1,2) and at least one other LRRC8 subunit (LRRC8B to -E) (1). Depending on the subunit composition, VRACs can conduct a wide range of organic compounds (3,4). Pancreatic islets prominently express LRRC8A and LRRC8D, subunits of volume-regulated VRAC anion channels. Given that constitutive LRRC8A knockout mice display high pre- and post-natal lethality, we deleted LRRC8A in pancreatic β-cells and investigated the role of VRAC in insulin secretion. We could elicit VRAC currents in β-cells by glucose- metabolism induced cell swelling. We showed that glucose-induced excitation and Ca^{2+} responses were delayed in onset, but not abolished, in β-cells lacking the essential VRAC subunit LRRC8A. Furthermore, first-phase glucose-induced insulin secretion was reduced. Mice lacking VRAC in β-cells had normal resting serum glucose levels but impaired glucose tolerance. We propose that opening of LRRC8/VRAC channels increases glucose sensitivity and insulin secretion of β-cells synergistically with KATP closure. Neurotransmitter-permeable LRRC8D-containing VRACs might have additional roles in autocrine/paracrine signaling within islets. VRAC, by depolarizing β-cells, enhances glucose sensing and insulin secretion (5).

Ionic modulation of immune checkpoint proteins

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Poster board 17

Despite extensive basic and clinical research on immune checkpoint regulatory pathways, little is known about the effects of the ionic tumour microenvironment on immune checkpoint expression and function. Here, we describe a mechanistic link between Na^+/K^+ ATPase inhibition and activity of indoleamine-2',3'-dioxygenase (IDO1), a well-characterised immune checkpoint. To assess IDO1 function, we employed a medium-high throughput screening assay based on spectrophotometric detection of kynurenine, a downstream tryptophan metabolite. Using this assay, we tested a library of 31 model ion channel targeting compounds. We discovered that the cardiac glycosides ouabain and digoxin inhibit kynurenine production in both lung (A549) and breast (MDA-MB-231) cancer cells with a moderate impact on cell survival. Na^+/K^+ ATPase inhibition by ouabain, but not digoxin, in both cell lines downregulated IDO1 expression. Ionic modulation via the Na^+/K^+ ATPase is a novel regulatory pathway of the immune checkpoint protein IDO1, with both mechanistic and clinical implications.

Poster Abstracts

In vitro inflammatory knee pain: Of Mice and Men

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Poster board 18

The ongoing pain associated with arthritis reduces the quality of human life. Injection of complete Freund's adjuvant (CFA) into the knee of mice induced inflammation and a concomitant decrease in their natural digging behavior within 24-hours. After inflammation, retrogradely labelled knee-innervating dorsal root ganglion neurons showed a decreased action potential threshold and increased transient receptor potential vanilloid-1 (TRPV1) expression (validated with immunohistochemistry). Subsequently, systemic administration of a TRPV1 antagonist normalized mouse digging behavior within 30-minutes, likely due to inhibition of pain signaling. To test the translational potential of these results, we recorded from mouse sensory neurons incubated overnight with synovial fluid from humans with osteoarthritis experiencing chronic pain (OA-SF). We find that OA-SF directly sensitizes mouse knee neurons by decreasing the action potential threshold and increasing the resting membrane potential implicating voltage-gated sodium (Na_V) and potassium (K_V) channels. Using Ca^{2+}-imaging we also find that OA-SF increases TRPV1 and TRPM8 activity compared to synovial fluid from healthy individuals, highlighting the importance of TRP channels in OA pain. Taken together, our data suggests that inflammatory mediators in OA-SF excite sensory neurons and hence drive knee pain. This *in vitro*, translational model can be incorporated into clinical trials to unbiasedly assess the efficacy of drugs on sensory neurons and hence identify drivers of OA pain.

K_V3 Modulators impact prefrontal cortex parvalbumin neuron function *ex-vivo*

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Poster board 19

Modulation of $K_V3.1/3.2$ channels expressed by cortical fast spiking neurons is hypothesized to be therapeutic in diseases such as Schizophrenia and Autism Spectrum Disorder. Here we tested the impact of Kv3 positive allosteric modulator AUT-1 on excitability and synaptic outputs of parvalbumin-expressing (PV) neurons of the adult mouse prefrontal cortex (PFC). First, in ex-vivo brain slices prepared from a PV-Cre::LoxP-TdTomato reporter mouse (postnatal week 8-12), we assessed the effect of bath application of AUT1 (10 µM) and potassium channel blocker TEA (300 µM-1mM) on evoked action potential generation in layer 2/3 PV neurons. We found that both compounds reliably and reversibly decrease the number of action potentials evoked by brief suprathreshhold current injections. TEA, but not AUT-1 efficacy is related to broadening of the action potential waveform. Next, we examined the impact of AUT1/TEA on $GABA_A$-mediated synaptic transmission from PV neurons onto postsynaptic pyramidal neurons (PNs), again in layer2/3 PFC. PV-Cre mice (ca. 6 weeks postnatal) were given local PFC stereotactic injection of an AAV encoding the optogenetic activitor chronos-GFP, the expression of which is Cre-dependent. After 3-5 weeks, slices were prepared and inhibitory post synaptic currents (IPSCs) recorded in PNs, elicited by brief (<0.5ms) flashes of blue light. We found that bath perfusion of AUT1 reversibly decreases IPSC amplitude, an effect reversed by subsequent TEA wash-in. Both effects are likely driven by presynaptic mechanisms, as suppression or enhancement of IPSC amplitude is accompanied by bidirectional alterations in synaptic integration during delivery of trains (50 Hz) or pairs (20 Hz) of synaptic stimuli. Together these data indicate that K_V3 channel modulation has complex actions on PFC circuits involving PV neurons, possibly due to compound mode-of-action and divergent outcomes of modulating of somatodendritic vs. axonal channels.

Poster Abstracts

E_{ACT} increases intracellular calcium levels by a TMEM16A-independent mechanism

Sarah Lilley
University of Sussex

Poster board 20

The N-aroylaminothiazole E_{ACT} was first described by Namkung et al. (2011) as an activator of the calcium-activated chloride channel, TMEM16A. Subsequently, E_{ACT} has been used as a tool compound by investigators to describe a wide variety of putative physiological functions of TMEM16A. The aim of the present study was to compare the pharmacology of E_{ACT} with alternative potentiators of TMEM16A that have been recently discovered by high-throughput screening.

Consistent with the original reported pharmacology, E_{ACT} increased anion secretory responses in models of epithelial ion transport that could be attenuated with the TMEM16A blocker, Ani9. Similarly, novel TMEM16A potentiators from 3 structurally distinct chemical series also increased the Ani9-sensitive anion secretion in these ion transport models.

To understand the mechanism of activation of these anion secretory currents, the effects of E_{ACT} and the novel TMEM16A potentiators on levels of intracellular calcium ($[Ca^{2+}]i$) were evaluated. The acute addition of E_{ACT} to primary CF-HBE induced a concentration-dependent increase in $[Ca^{2+}]i$. Pre-treatment of cells with Ani9 had no effect on the E_{ACT}-induced rise in $[Ca^{2+}]i$. In contrast, the novel TMEM16A potentiators had no effect on $[Ca^{2+}]i$.

The observation that E_{ACT} could increase $[Ca^{2+}]i$ questioned the reported pharmacological mechanism of TMEM16A activation by this molecule ie. via a direct interaction with the channel. To address whether E_{ACT} could directly activate TMEM16A in the absence of an elevation of $[Ca^{2+}]i$, patch-clamp studies were performed under conditions of buffered $[Ca^{2+}]i$. Under these conditions, with $[Ca^{2+}]i$ tightly clamped, E_{ACT} showed no evidence of any activity on TMEM16A. In contrast, the novel TMEM16A potentiators all showed a potent increase in channel function.

Together, these data do not support the description of E_{ACT} as a direct TMEM16A modulator but are consistent with its activation of TMEM16A being indirect, the result of an as yet undefined mechanism leading to an elevation of $[Ca^{2+}]i$. Furthermore the recent proposal that TMEM16A positively regulates $[Ca^{2+}]i$ (Cabrita et al., 2017) is not consistent with the lack of effect of either the TMEM16A blocker Ani9 or the novel potent and selective TMEM16A potentiators on $[Ca^{2+}]i$. Our data suggest that literature reports of TMEM16A function that have relied on the use of E_{ACT} as a pharmacological tool should be interpreted with caution.

Namkung et al (2011) FASEB J 25(11):4048-4062

The pharmacology of novel TMEM16A potentiator compounds

Henry Danahay
University of Sussex

Poster board 21

TMEM16A was recently identified as a calcium-activated chloride conductance and a key orchestrator of anion secretion in the human airway epithelium (Caputo et al 2008; Schroeder et al 2008; Yang et al 2008). It is now clinically established that promoting anion secretion in the airway leads to enhanced mucus clearance and reduced exacerbation frequency in CF patients and as such TMEM16A represents an important target for the next generation of mucokinetics. Importantly, positive regulators of TMEM16A function will be expected to be of benefit in all CF patients, irrespective of their CFTR mutational status.

Using 4 parallel screening approaches, we identified several chemically diverse, low molecular weight compounds that potentiated TMEM16A function. These hit compounds were validated for TMEM16A function using a patch-clamp assay under conditions where $[Ca^{2+}]i$ was tightly buffered at an EC20 for TMEM16A channel activity. This enabled hits that activated TMEM16A by non-specifically elevating $[Ca^{2+}]i$ to be rapidly filtered out from the hit list.

The efficacy of *bona fide* TMEM16A potentiators translated through to function in ion transport studies in CF-HBE. Pre-treatment of CF-HBE with TMEM16A potentiators for between 5 min to 96h resulted in an enhancement of Ca^{2+}-mediated anion-secretory responses that were sensitive to the TMEM16A blocker, Ani9. Measurements of $[Ca^{2+}]i$ confirmed that TMEM16A potentiators had no effect on calcium mobilization, consistent with a direct effect on the channel.

A Series 1 TMEM16A potentiator, ETX001, increased the secretion of airway surface liquid (ASL) in CF-HBE. The ETX001-driven increase in ASL height was further enhanced in cells that had been pre-treated with IL-13 to boost TMEM16A expression. A close structural analogue of ETX001, ETX002, that is inactive on TMEM16A, did not increase ASL height.

Together, these data support the concept that potentiators of the alternative airway chloride conductance, TMEM16A, can restore anion conductance and fluid secretion in both primary CF cells. Enterprise Therapeutics are advancing TMEM16A potentiators into clinical development.

Caputo et al (2008) Science 322(5901):590-594
Schroeder et al (2008) Cell 134(6):1019-1029
Yang et al (2008) Nature 455(7217):1210-1215

Poster Abstracts

Adaptive voltage protocols increase precision of voltage-gated ion channel measurements on high-throughput automated patch clamp platforms

Sarah Williams
Charles River Laboratories

Poster board 22

Voltage-gated sodium (Na_V) channels are studied extensively due to their potential as targets for several indications, such as pain, epilepsy, cardiac and muscle paralysis. Some Na_V channel modulators show state-dependence and bind preferentially to the inactivated state of the channel. The potency of state-dependent compounds are known to vary depending on the percentage inactivation of the channels.
To calculate accurate compound activity the precise value for the V_{half} of inactivation should be used for each cell. The adaptive protocol block for the Sophion Qube 384-well automated patch clamp platform has made it possible to separately define the voltage applied to each individual well for both the activation and inactivation of the channels, enabling the generation of more precise data for voltage-gated ion channels.
The incorporation of the adaptive protocol did not change the performance of our $Na_V1.1$ assay compared to the standard protocol. The adaptive protocol significantly decreased the variability of the percent current inactivation: in the standard protocol experiment approximately 80% of the wells had percent current inactivation between 26-66%, whereas in the adaptive protocol experiment the percent current inactivation was between 47-61% for 80% of the wells. A range of known state-dependent compounds were tested as concentration-response curves against $Na_V1.1$ in both protocols, with compound potencies found to be similar. However, the compound data at 10 µM was found to be much less variable in the adaptive protocol experiment, and in a high throughput screen this reduced variability should lead to increased confidence in the results. For Amitriptyline, the variation in percent inhibition at 10 µM was between 16-62% against $Na_V1.1$, which may mean that in some cases the compound would not have been detected as a 'hit'.
In summary, the new adaptive protocol enables increased control of the state that voltage-gated channels during an experiment on a 384-well high throughput automated patch clamp platform, which leads to reduced data variability and increased confidence in compound testing results.

Development and validation of NMDA ligand-gated ion channel assays using the Qube 384 automated electrophysiology platform

Abigail Marklew
Charles River Laboratories

Poster board 23

Ligand-gated ion channels are of particular interest to the pharmaceutical industry for the treatment of diseases from a variety of therapeutic areas including CNS disorders, respiratory disease and chronic pain. Ligand-gated ion channels have historically been investigated using fluorescence-based and low throughput patch-clamp techniques. However the development of the Qube 384 automated patch-clamp system has allowed rapid exchange of liquid and direct measurement of ion channel currents on a millisecond timescale, making it possible to run HTS campaigns and support SAR with a functional readout.

Here, we have used the Qube platform to develop an assay against the NR1/NR2A receptor, which is part of the N-methyl-D-aspartate (NMDA) glutamate receptor family. For this assay we utilized stacked liquid addition which enabled us to assess the open state kinetics of the channel and to investigate the effects of antagonists with multiple modes of actions.

We observed stable agonist responses for both NMDA and glycine, with EC_{50} values comparable to literature values. The assay format was stable over multiple agonist applications and wash periods, which meant it was suitable for compound testing and sensitive enough to detect antagonists with multiple modes of action. D-AP-5, a competitive antagonist, showed stable NR1/NR2A receptor over six consecutive agonist/antagonist applications. The potency of ketamine, a use-dependent inhibitor, increased over the six agonist/antagonist applications. By utilizing of stacked liquid additions in a 384 automated electrophysiology platform we have created an assay against NMDA receptors which is suitable for compound testing and sensitive enough to detect different modes of actions.

Presentation Abstracts

Cellular and molecular basis of mechanosensory adaptations in tactile specialist birds

Slav Bagriantsev
Yale University

Mechanically gated ion channels are essential for the detection of touch by peripheral sensory neurons, but many aspects of tactile physiology and mechanotransducer function remain obscure. We employ electrophysiology, histology and transcriptomics to reveal cellular and molecular variations that accompany the transition from visual to tactile foraging in birds, at the level of somatosensory neurons. We show that mechanosensory potentiation is accompanied by a proportional expansion of trigeminal mechanoreceptors at the expense of neurons that sense temperature and pain. On the biophysical level, mechanoreceptors from tactile specialists express the Piezo2 channel with enhanced ability to convert mechanical stimulation into excitatory current. Our studies reveal cellular and molecular mechanisms that drive mechanosensory specialization in vertebrates in an organ-specific manner.

TREK-1 activation to solve the opioid crisis?

Sylvie Ducki
Institut de Chimie de Clermont-Ferrand

Chronic pain affects 1.5 billion people worldwide, causing a great deal of discomfort among patients and an enormous economic and societal burden. Inadequate pain control, undesirable side-effects associated with current analgesics as well the recent opioid crisis have revived interest in analgesic drug development. The challenge is to develop original analgesics with novel modes of action to address the unmet needs of patients.
Devilliers reported that TWIK-Related K^+ channel TREK-1 -/- mice were more sensitive than wild-type TREK-1 +/+ mice to painful stimuli, suggesting that activation of TREK-1 could result in pain inhibition.
Various approaches of drug discovery were explored in order to develop original analgesic drugs targeting TREK-1.

1. Rodrigues, N. ; Bennis, K. ; Vivier, D. ; Pereira, V. ; Chatelain, F. C. ; Chapuy, E. ; Deokar, H. Busserolles, J. ; Lesage, F. ; Eschalier, A. ; Ducki, S. "Synthesis and structure-activity relationship study of substituted caffeate esters as antinociceptive agents modulating the TREK-1 channel" European Journal of Medicinal Chemistry 2014, 75, 391-402.
2. D. Vivier, K. Bennis, F. Lesage, S. Ducki "Perspectives on the Two-Pore Domain Potassium Channel TREK-1 (TWIK-Related K+ Channel 1). A Novel Therapeutic Target?", Journal of Medicinal Chemistry 2016, 59, 5149-5157
3. D. Vivier, I. Ben Soussia, N. Rodrigues, S. Lolignier, M. Devilliers, F. C.

Resin-acid derivatives open potassium channels via the voltage-sensor domain – a putative anti-seizure action

Fredrik Elinder
Linköping University

Voltage-gated ion channels are major targets for pharmaceutical drugs in diseases with increased electrical excitability, such as epilepsy, cardiac arrhythmia, and pain. While most known drugs block sodium or calcium channels, opening of voltage-gated potassium channels is a much less explored alternative. Here I report on the action of resin-acid derivatives on the voltage gated M-type potassium channel (K_V7.2/7.3). They bind to the voltage-sensor domain, in the cleft between the transmembrane segments 3 and 4, facing the lipid bilayer. From this position, the negatively charged carboxyl group (or other groups) attracts the positively charged voltage sensor S4 to its activated up state. This pulls the gate open to allow potassium to flow out from the cell, hyperpolarize the neuron, and dampen excitability. Modification of the charge has large effect on the channel-opening capacity. By extending the stalk on which the charge is connected to the rest of the compound, the effect can be dramatically increased. However, a stalk longer than three atoms makes the compounds almost without effect. Some compounds seem to be selective for the K_V7.2/7.3 channel and have anti-seizure actions in a simple zebrafish larvae epilepsy model.

Presentation Abstracts

Ion Channels in Immunity: CRAC channels and beyond

Stefan Feske
New York University

Ion channels and transporters (ICTs) control ion fluxes across lipid membranes and play pivotal roles in a multitude of cell functions. While ICTs have been extensively investigated in excitable cells such as neurons, much less is known about the role of ICTs in the function of immune cells and immunity. Of the hundreds of ICTs only 10-15 are well established to play a role in immune responses. This includes the Ca^{2+} release activated Ca^{2+} (CRAC) channel encoded by ORAI and STIM genes. Our lab has shown that CRAC channel function is essential for T cell mediated immunity to infection and in autoimmunity, in part by regulating gene expression programs in proinflammatory and regulatory T cells. In particular, metabolic pathways such as glycolysis and mitochondrial respiration that control T cell proliferation and effector functions are regulated by Ca^{2+} influx through CRAC channels. Besides CRAC channels, other ICTs conducting Ca^{2+}, Mg^{2+}, Zn^{2+}, K^+ and Cl^- such as TRPM7, MAGT1, ZIP7, $K_V1.3$ and LRRC8A, respectively, have been shown to regulate immune cell function. Overall however, the knowledge regarding ICTs in immune function is limited. My lab has developed in vivo forward genetic screening approaches to identify novel ICTs that mediate T cell function in immunity to infection, tumors and in autoimmune diseases. This talk will discuss new insights into the role of ion channels in T cell mediated immune function.

Regulating pathological cardiac remodeling via TRPC channels and new players in the endo-lysosomal Ca^{2+} signaling

Marc Freichel
University of Heidelberg

The development of pathological cardiac remodeling is characterized by alterations in the intracellular calcium homeostasis in cardiomyocytes, and several ion channels and regulatory proteins thereof contribute to this process. We are interested in calcium signaling pathways, which mediate processes leading to the development of pathological remodeling but do not affect the beat to beat contractile function in the healthy heart. Receptor- and store-operated calcium entry pathways may act in this way and were previously described in cardiac cells [1]. Amongst the TRP channeldependent calcium entry pathways, of which many were described in cardiomyocytes [2], the TRPC1/TRPC4-mediated background calcium entry pathway (BGCE) belongs to these signaling pathways [3]. BGCE was recently identified in adult ventricular cardiomyocytes and is enhanced by neurohumoral stimulation with catecholamines and Angiotensin II, respectively. Inactivation of TRPC1/TRPC4-mediated BGCE in vivo does not affect cardiac contractility in untreated mice but leads to significant reduction of cardiac hypertrophy, fibrosis and maladaptive gene expression, and protects from the development of cardiac disfunction after chronic pressure overload in the transverse aortic constriction (TAC) model. During our search for new proteins, that may modulate calcium homeostasis in cardiomyocytes, we have identified new proteins that operate as regulators for calcium release from intracellular acidic organelles and were, therefore, termed OCaRs. In my talk, the contribution of these proteins for cardiomyocyte calcium homeostasis, cardiac function and development of cardiac remodeling processes will be presented.

1. Freichel, M. et al. Store-operated cation channels in the heart and cells of the cardiovascular system.Cell Physiol Biochem 9: 270–283 (1999).
2. Freichel M, Berlin M, Schürger A, Mathar I, Bacmeister L, Medert R, Frede W, Marx A, Segin S, Londoño JEC. TRP channels in the heart. In: Emir TLR, ed. Neurobiology of TRP channels. 2nd ed, Boca Raton (FL): CRC Press/Taylor & Francis, Chapter 9 (2017).
3. J.E. Camacho Londono et al., A background Ca2+ entry pathway mediated by TRPC1/TRPC4 iscritical for development of pathological cardiac remodeling, European Heart Journal 36: 2257-2266 (2015).

Structural basis of toxin binding to voltage-gated sodium channels and what it teaches us about voltage-gating mechanisms

David Hackos
Genentech

Voltage-gated sodium (Na_V) channels are responsible for the generation and propagation of action potentials and thereby play an essential role in the functioning of electrically-excitable cells. They also represent important drug targets since modulating them in specific and selective ways could be effective in several neurological disorders including pain, cardiovascular disease, epilepsy, schizophrenia, Alzheimer's disease, etc. Unfortunately, selective Nav channel modulators have been difficult to identify, in part due to an incomplete molecular understanding of binding sites that could enable molecularly selective pharmacology. Using a combination of X-ray crystallography and cryo-electron microscopy techniques, we have recently solved structures of the spider toxin binding site on the second voltage-sensing domain (VSD2) (Xu et al., Cell 2019) as well as the scorpion toxin binding site on VSD4 (Clairfeuille et al., Science 2019). These structures not only reveal the molecular details of these pharmacologically important binding sites, but also reveal details about how voltage-sensors move across the membrane electric field and how these movements couple to the activation and inactivation machinery.

Presentation Abstracts

Autoantibodies in the human brain: how immunology upsets neurons

Sarosh Irani
University of Oxford

Human autoantibodies which target the extracellular domains of native CNS proteins are pathogenic in a variety of human illnesses including forms of epilepsy, encephalitis and demyelination. Common targets of these autoantibodies include leucine-rich glioma inactivated 1 (LGI1), contactin associated protein like 2 (CASPR2) and the NMDA, GABAA/B and AMPA receptors. Patients with an autoantibody which targets one of these proteins have highly-distinctive syndromes. For example, patients with LGI1 antibodies have frequent seizures and amnesia. Furthermore, intraventricular infusion of these antibodies into experimental animals has been shown to reproduce memory deficits. To explore the underlying molecular mechanisms, we generated patient-derived monoclonal antibodies (mAbs) against LGI1, which is traditionally divided into leucine-rich repeat (LRR) and epitempin repeat (EPTP) domains. Sera (30/30) and CSF (8/10) bound both domains with stable LRR- and EPTP-specific titers over time. In contrast to this serum and CSF polyclonality, the 14 mAbs from both patients recognized either the LRR or EPTP domain. Sequence analysis revealed genetic heterogeneity and high mutation frequencies across both specificities. LRR-specific mAbs recognized LGI1 bound to its known interaction partners, a disintegrin and metalloproteinase domain-containing protein (ADAM) 22 and ADAM23, and induced internalization of the LGI1-ADAM22/23 complex in both HEK293T cells and live hippocampal neurons. In contrast, EPTP-specific mAbs inhibited the docking of LGI1 onto ADAM22/23. Taken together, the use of mAbs to dissect serum polyreactivities has revealed distinct LGI1-antibodies, which target the two major domains of LGI1, and have dichotomous molecular mechanisms, both with pathogenic potential.

The ClC-2 chloride channel and its role in aldosterone secretion

Thomas J. Jentsch
Max-Delbrueck-Center Berlin

ClC-2 is a plasma membrane Cl^- channel that belongs to the CLC family of Cl^-- channels and $2Cl^-/H^+$-exchangers. It is very widely expressed in mammalian tissues and is activated by hyperpolarization and cell swelling. ClC-2 plays a role in various human pathologies. We have previously shown that disruption of ClC-2 in mice leads to blindness and male infertility 1 as well as leukodystrophy 2. Similar phenotypes were later detected in human patients with CLCN2 loss-of-function mutations. In astrocytes, ClC-2 associates with the cell adhesion molecule GlialCAM 3 which changes its localization and biophysical properties. Mutations in GliaCAM, as well as in MLC1 with which both proteins form a tertiary complex, also cause leukodystrophy in humans and mice 4. Together with the group of M.C. Zennaro (Paris), we have recently shown that an activating mutation in ClC-2 result in human juvenile hyperaldosteronism which is associated with severe hypertension 5. This mutation, as well as other aldosteronism-causing mutations identified by others 6, affect residues in a region of ClC-2 we had previously identified to be crucial for channel gating 7. We showed that these mutations dramatically (>10-fold) increase ClC-2 currents in heterologous expression and increase aldosterone production when expressed in a cell line of aldosterone-producing glomerulosa cells.To demonstrate that genetic 'opening' of ClC-2 suffices to cause hyperaldosteronism, we now generated a KI mouse carrying an artificial mutation that opens ClC-2. These mice indeed display high aldosterone, low renin, and high blood pressure. Patch-clamp of glomerulosa cells in situ shows that these are strongly depolarized. This leads to an increase in intracellular Ca^{2+} and induction of aldosterone synthase. Our KI mice are an excellent novel mouse model for human hyperaldosteronism.

1. Bösl, M. R. et al. Male germ cells and photoreceptors, both depending on close cell-cell interactions, degenerate upon ClC-2 Cl--channel disruption. EMBO J 20, 1289-1299 (2001).
2. Blanz, J. et al. Leukoencephalopathy upon disruption of the chloride channel ClC-2. J Neurosci 27, 6581-6589 (2007).
3. Jeworutzki, E. et al. GlialCAM, a protein defective in a leukodystrophy, serves as a ClC-2 Cl- channel auxiliary subunit. Neuron 73, 951-961 (2012).
4. Hoegg-Beiler, M. B. et al. Disrupting MLC1 and GlialCAM and ClC-2 interactions in leukodystrophy entails glial chloride channel dysfunction. Nature communications 5, 3475, doi:10.1038/ncomms4475 (2014).
5. Fernandes-Rosa, F. L. et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. Nat Genet 50, 355-361, (2018).
6. Scholl, U. I. et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. Nat Genet 50, 349-354 (2018).
7. Gründer, S., Thiemann, A., Pusch, M. & Jentsch, T. J. Regions involved in the opening of CIC-2 chloride channel by voltage and cell volume. Nature 360, 759-762 (1992).

Presentation Abstracts

Molecular conversion of presynaptic P2X receptor subtype at brainstem synapse

Fusao Kato
Jikei University

Presynaptic terminals are the key site for the precise regulation of neuron-to-neuron messaging in the brain. Though they are extremely small in size, they are rich in various types of molecules including ion channels underlying presynaptic regulation of transmitter release. P2X receptor channels are extracellular ATP-gated channels highly permeable to Ca^{2+} at depolarized, i.e., at resting, membrane potentials (inwardly rectifying), unlike the NMDA receptors. In brain synapses, they play a role as a converter from extracellular ATP to transmitter release even in the absence of presynaptic action potentials (Shigetomi and Kato, 2004). P2X receptors are composed of three hetero/homo-subunits. Unfortunately, there is a confusion in the nomenclature of subunits and receptor subtypes. For example, P2X2 is a subunit name and also a homomeric channel name composed of three P2X2 subunits. I propose to call the latter as P2X2/2/2. In most of the brain structures, molecular manipulation of the presynaptic molecules is challenging because of their size and location. Here I show an example of an effect of in-vivo gene knockdown in the presynaptic neurons that changed the transmitter release phenotype in the central terminal measured ex-vivo from the P2X2/3/3 to P2X2/2/2.

Drug development strategies of $Na_V1.7$ blocker

Sung-Young Kim
Daewoong Pharmaceutical Co., Ltd.

Chronic pain represents a critical unmet medical need that afflicts over one hundred million Americans with an extremely high economic cost. But current available therapies lack robust efficacy, carry significant abuse potential, and/or suffer from low tolerability and safety. $Na_V1.7$ controls the passage of sodium ions into sensory neurons, and the target garnered attention after it was found that rare human mutations characterized by a loss of function in $Na_V1.7$ channels resulted in congenital insensitivity to pain. Despite genetic validation, a selective $Na_V1.7$ blocker PF-05089771 failed to demonstrate efficacy in clinical trials. PF-05089771 is a potent and selective $Na_V1.7$ blocker, but it lacks enough target engagement to exhibit efficacy. Looking at current developments, there are various strategies to target $Na_V1.7$ including small molecule, biologics. In this presentation, I will introduce strategies of various $Na_V1.7$ blocker for pain drug development.

Phosphoinositides modulate the voltage dependence of Two-Pore Channel 3

Yoshihiro Kubo
National Institute for Physiological Sciences

Two-Pore Channels, or Two-Pore Na^+ Channels (TPCs), contain two domains of a functional unit of voltage-dependent cation channels. Each domain is considered to be responsible for phosphoinositide (PI) binding or for voltage sensing. Among the three members of the TPC family, TPC1 and TPC2 are activated by PI(3,5)P2, while TPC3 has been understood not to be affected by any PIs. Here, we report that TPC3 has a sensitivity to PI(3,4)P2 and PI(3,5)P2, but not to PI(4,5)P2, and that the extremely slow increase in TPC3 currents by depolarization in Xenopus oocytes is due to the production of PI(3,4)P2. Similarly to TPC1, the cluster of basic amino acid residues in domain I is critical for PI sensitivity, but with a slight variation possibly allowing TPC3 to be sensitive to both PI(3,4)P2 and PI(3,5)P2. We also newly found that TPC3 has a unique PI-dependent modulation mechanism of voltage dependence, which is achieved by a specific bridging interaction between domain I and domain II. Taken together, these findings show that TPC3 is a unique member of the TPC family that senses PIs and has a strong coupling between PI binding and voltage-dependent gating.

Ion channel gene therapy for refractory epilepsy

Dimitri Kullmann
UCL Institute of Neurology

Epilepsy is common and 25-30% of affected individuals continue to experience seizures despite optimal medication. Conventional anti-seizure drugs mainly work by reducing excitation or promoting inhibition, but have a limited therapeutic index. First, the entire brain is exposed, and not only the epileptogenic zone. Second, drugs generally act indiscriminately on excitatory and inhibitory neurons. Gene therapy offers the opportunity for treatment to be targeted to a specific region of the brain, and, by use of cell-type specific promoters, to bias expression of therapeutic transgenes to specific cell types. I shall summarise work showing successful suppression of seizures in rodent models of epilepsy by overexpressing the potassium channel $K_V1.1$ in excitatory neurons, and by using optogenetics and chemogenetics. I shall also describe an autoregulatory strategy that uses a glutamate-gated chloride channel to suppress seizures in response to pathological elevations of glutamate. Some of these strategies are ready for clinical translation

Presentation Abstracts

SK channel regulation of synaptic plasticity

Jack Mellor
University of Bristol

Synaptic plasticity is a fundamental process underpinning the encoding and consolidation of memory. It critically depends on postsynaptic calcium signalling within individual dendritic spines. These calcium signals primarily arise from voltage-dependent NMDA receptors and calcium channels which are regulated by ionic conductances. We have focussed on one particular conductance – SK calcium activated potassium channels – which have the unique property of being activated by calcium and hyperpolarise the postsynaptic membrane inhibiting NMDA receptors and calcium channels. These properties endow individual synapses with intrinsic negative feedback control of calcium concentrations which are finely tuned and controlled by SK channel activity. We have found that SK channels are inhibited by both muscarinic M1 receptors and metabotropic glutamate receptor group 1 receptors (mGluR1) in the hippocampus causing a relief of NMDA receptor regulation and increased calcium influx in response to synaptic stimulation. This effectively enables M1 and mGluR1 receptors to "gate" the induction of synaptic plasticity. We demonstrate this using a combination of in vitro electrophysiology, 2-photon calcium imaging and 3-D biophysical modelling of spine dynamics. We propose that M1 and mGluR1 receptors inhibit SK channels via a common signalling pathway but that each are engaged during distinct phases of learning. M1 receptors provide the window of synaptic plasticity for initial learning whereas mGluR1 enables synaptic plasticity during memory consolidation.

This work was funded by Wellcome Trust, BBSRC, MRC, EPSRC and Eli Lilly & co.

Insights into understanding and developing modulators of GABA-A receptors

Paul Miller
Cambridge University

γ-aminobutryic acid Type-A receptors ($GABA_ARs$) are the principle mediators of rapid inhibitory neurotransmission in the brain and the targets of many endogenous, experimental and clinical ligands. I will present recent structures that reveal insights into the modes of action of benzodiazepines at $GABA_ARs$. I will also present structural and functional data from investigations using novel protein modulators, nanobodies, some of which behave as positive allosteric modulators of $GABA_ARs$. These possess higher selectivity than traditional small molecules and should be useful as improved investigational tools, and perhaps one day lead to novel clinical drugs.

Juvenile NMDA receptors containing the GluN3A subunit: gate keepers of synapse plasticity, cognition and brain disease

Isabel Pérez-Otaño
Alicante Institute of Neuroscience

NMDA receptors are glutamate-gated ion channels with central roles in brain development, synapse plasticity and memory. They assemble as heterotetrameric combinations of 3 types of subunits (GluN1, GluN2A-D, and GluN3A-B), which functional expression varies across brain regions and developmental stages to fulfill unique functions. GluN3A-containing NMDA receptor subtypes (GluN3A-NMDARs) are predominantly expressed in the central nervous system during a temporal window of early postnatal development that ends with the emergence of mature neuronal circuits and adaptive behaviours. GluN3A-NMDARs have non-conventional biophysical properties compared to classical NMDAR subtypes and seem to have an important role in delaying synapse maturation until the arrival of sensory experience and in targeting non-used synapses for pruning. Precisely how and why remain poorly understood. Recent work suggests that reactivation of GluN3A expression at inappropriate ages may underlie maladaptive synaptic rearrangements observed in addiction, neurodegenerative disease, and other major brain disorders. I will discuss current evidence for these and other emerging roles for GluN3A-NMDARs in the physiology and pathology of the central nervous system, and newly discovered mechanisms that support their function.

Presentation Abstracts

Migraine-associated TRESK mutations increase neuronal excitability through alternative translation initiation and inhibition of TREK

Guillaume Sandoz
Université de Nice Sophia Antipolis

It is often unclear why some genetic mutations to a given gene contribute to neurological disorders while others don't. For instance, two mutations have previously been found to produce a dominant negative for TRESK, a two-pore-domain K^+ channel implicated in migraine: TRESK-MT, a 2 bp frameshift mutation, and TRESK-C110R. Both mutants inhibit TRESK, but only TRESK-MT increases sensory neuron excitability and is linked to migraine. Here we identify a new mechanism termed frameshift mutation-induced Alternative Translation Initiation (fsATI), that may explain why only TRESK-MT is associated with migraine. fsATI leads to the production of a second protein fragment, TRESK-MT2, which co-assembles with and inhibits TREK1 and TREK2, two other two-pore-domain K^+ channels, to increase trigeminal sensory neuron excitability leading to a migraine-like phenotype in rodents. These findings identify TREK1 and 2 as potential molecular targets in migraine and suggest that fsATI should be considered as a distinct class of mutations.

A Pharmacological Master Key Mechanism that Unlocks the Selectivity Filter Gate in K+ Channels

Marcus Schewe
Université de Nice Sophia Antipolis

Potassium channels (K^+) have been evolutionarily tuned for activation by diverse biological stimuli, and pharmacological activation is thought to target these specific gating mechanisms. Here we report a class of negatively charged activators (NCAs) that bypass the specific mechanisms but act as master keys to open K^+ channels gated at their selectivity filter (SF), including many K2P channels, voltage-gated hERG channels and Ca^{2+}-activated BK type channels. Functional analysis, X ray crystallography and molecular dynamics (MD) simulations revealed that the NCAs bind to similar sites below the SF, increase pore and SF K^+ occupancy and open the filter gate. These results uncover an unrecognized poly-pharmacology among K^+ channel activators and highlight a filter gating machinery that is conserved across different families of K^+ channels with implications for rational drug design.

Function and modulation of axonal K_V7 channels in hippocampal neurons

Mala Shah
University College London

Axons express a variety of ion channels which play a fundamental role in modulating action potential initiation, shapes and propagation and, thereby, neuronal firing patterns. In this talk, I will present evidence that the axon initial segment of hippocampal pyramidal neurons and granule cells express K_V7 channels, where they elevate the action potential threshold and reduce neuronal excitability. In addition, I will present our recent work that shows that these channels are also located in granule cell axon (mossy fiber) giant synaptic terminals and affect synaptic release. Further, since K_V7 channel activity is modified by neurotransmitters such as acetylcholine, I will discuss how axonal K_V7 channels are affected by neurotransmitters and the implications of this for neuronal function.

TRPC1 as a negative regulator for TRPC4 and TRPC5 channels

Insuk So
Seoul National University

Transient Receptor Potential Canonical (TRPC) channels are calcium-permeable, non-selective cation channels with wide tissue-specific distribution. Among 7 TRPC channels, TRPC 1/4/5 and TRPC3/6/7 are subdivided based on amino acid sequence homology. TRPC4 and TRPC5 channels exhibit cationic current with homotetrameric form but they also form heterotetrameric channel such as TRPC1/4 or TRPC1/5 once TRPC1 is incorporated. The expression of TRPC1 is ubiquitous whereas the expression of TRPC4 and TRPC5 are rather focused in nervous system. With the help of conditional knockout of TPRC1, 4 and/or 5 genes, TRPC channels made of these constituents are reported to be involved in various pathophysiological functions such as seizure, anxiety-like behavior, fear, Huntington's Disease, Parkinson's Disease and many others. In heterologous expression system, many issues such as activation mechanism, stoichiometry and relative cation permeabilites of homomeric or heteromeric channels have been addressed. In this sympoisum, we discussed the role of TRPC1 channel per se in plasma membrane, role of TRPC1 in heterotetrameric conformation (TRPC1/4 or TRPC1/5), and relationship between TRPC1/4/5 channels, calcium influx and voltage-gated calcium channels.

Presentation Abstracts

Structural and functional studies of vanilloid subtype TRP channels

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Transient receptor potential (TRP) channels are a superfamily of cation-permeable ion channels that are widely known for their role as transducers of sensory modalities. The TRPV subfamily of TRP channels (V is for "vanilloid", a chemical that activates members of this subfamily) includes six members, of which TRPV1-4 are temperature sensitive and TRPV5-6 are highly calcium selective. We studied TRPV6 using both X-ray crystallography and cryo-EM. We identified binding sites of permeant ions Ca^{2+} and Ba^{2+} and ion channel blocking ion Gd^{3+}. Ca^{2+} selectivity is determined by direct coordination of Ca^{2+} by a ring of aspartate side chains in the ion channel selectivity filter. Combined with molecular dynamics, our data suggest that ion permeation in TRPV6 occurs according to the knock-off mechanism. We solved TRPV6 structures in the open and closed states and proposed the mechanism of the TRPV6 channel opening, which is accompanied by an α-to-π helical transition in the pore-forming S6 segment. This activation gating process can be suppressed by the inhibitor 2-APB that induces TRPV6 channel closure by modulating protein-lipid interactions. Alternatively, TRPV6 can be inhibited via calmodulin (CaM)-mediated Ca^{2+}-induced inactivation. The TRPV6-CaM complex exhibits 1:1 stoichiometry; one TRPV6 tetramer binds both CaM lobes, which adopt a distinct head-to-tail arrangement. The CaM C-terminal lobe plugs the channel through a unique cation-π interaction by inserting the side chain of lysine K115 into a tetra-tryptophan cage at the pore's intracellular entrance. We also found that 2-APB, that acts as an agonist of TRPV3 channels, binds three allosteric sites distal to the TRPV3 channel pore. While 2-APB binding causes a similar to TRPV6 α-to-π helical transition in S6 of TRPV3, it results in much more significant transformations of S6 and TRP helix as well as of the overall shape of TRPV3. Our findings further extend knowledge of structure and function of TRPV channels that can aid in the design of drugs for the treatment of numerous diseases, including cancers, inflammatory skin conditions, itch, and pain.

Temperature-sensitive TRP channels as drug targets

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Transient receptor potential (TRP) channels form a superfamily of cation channels involved in a wide variety of physiological and pathophysiological processes. In particular, several mammalian TRP channels are expressed in sensory neurons, where they act as molecular sensors of thermal and chemical cues. Using single and combined mouse knockout models, we have investigated the role of various somatosensory TRP channels in acute pain sensation, and assessed how their expression and function changes in inflammatory conditions associated with hyperalgesia and ongoing pain. We further explore how pharmacological targeting of specific TRP channels may be developed to treat a variety of chronic pain conditions.

A 'Target-class' approach to identifying novel activators of K2P channels

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Two pore domain potassium (K2P) channels are a diverse family of potassium channels all known to possess a unique four transmembrane domain, two pore topology. They function to carry background potassium current and primarily act to maintain cellular resting membrane potential. There is accumulating genetic and functional evidence demonstrating a role for K2Ps in human pathophysiologies, most notably pain and migraine. However, identification of selective small molecules that activate K2Ps has proven difficult and this has in turn, limited efforts to develop K2P targeting therapeutics. Target-class drug discovery is a complementary approach to disease focused strategies which concentrate on either a single, defined and well validated target or rely on using a physiologically relevant phenotypic assay. Target-class discovery allows for the spreading of risk across a family of unprecedented targets, offering a greatly increased chance of identifying novel pharmacological agents by driving synergies in assay development, target structure and ligand development. We have developed an automated system for applying 'Target-class' discovery to the K2P superfamily, with the aim of identifying channel activators. The identification of ion channel activators can be compromised in cellular systems where the target is expressed at high levels, such as an over-expressing stable cell line. To avoid these issues, we used BacMam to express each K2P channel in mammalian cells. BacMam offers many advantages, including safety and reduced development time, compared to generating stable cell lines, but importantly it allows the precise titration of expression of the gene of interest. This enables the generation of cell reagents where we can select a level of K2P expression and function purposely designed and optimized for the identification of channel activators. Using this system in combination with a thallium flux assay, we screened a 10k representative set of the full LifeArc compound collection and a library of 1k FDA approved compounds against each of the K2P sub-families. Novel, selective activators were identified, and activity confirmed for a subset using electrophysiology. This 'target class' approach has allowed the simultaneous interrogation of a family of targets and identified a diverse range of previously undescribed pharmacology. Using novel automation and analysis techniques, screening synergies have been maximized and early phase hit identification timelines minimized. It has allowed the identification of K2P drug targets which are amenable to small molecule activation, de-risking multiple channels from a technical point of view and provided selective starting points for future chemical optimization.

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