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Ion Channels: Too Complex for Rational Drug Design?

Meeting Review

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Introduction

Beneath soaring eagles and snow-capped peaks, a Keystone Symposium was held from February 4–10, 1996, in Tamarron, Colorado. Under the banner, "Ion Channels as Therapeutic Targets," the organizers (Michael Cahalan, University of California, Irvine; K. George Chandy, University of California, Irvine; Doug Hanson, Pfizer; Alan North, Glaxo Institute) brought together scientists from academia and industry and dared us to admit to our successes and failures. At the heart of this challenge was a question: can drugs acting on ion channels be rationally designed? The driving motivation behind molecular-based drug design is clear: all drugs now marketed that act on ion channels were discovered empirically (either by clinical use for other indications or by broad functional screening) rather than by molecular insight, and most have been shown to have serious safety and efficacy problems. Granted, our new scientific methods are implicating ion channel dysfunction in some disease states and identifying new drug targets as well as new modes of drug action, but are we moving toward development of more successful ion channel modulating drugs? Here, we review the symposium presentations partitioned into three catagories: known targets and drugs in use, new targets and drug candidates, and new biology. The efforts of the organizers were rewarded with at least two tangible results: first, a foundation was laid for détente between accrued knowledge using older methods and newly emerging opportunities; second, the slopes remained barren and lecture hall full.

Known Targets and Drugs in Use

Sulfonylurea Receptors, ATP-Sensitive K^+ Channels, the Pancreas, and Beyond

Sulfonylureas are a class of drugs used to treat non-insulin-dependent diabetes mellitus. They act by binding to sulfonylurea receptors (SURs), members of the ABC transporter family that are present on pancreatic β cells. Drug binding inhibits ATP-sensitive K^+ channels (K_{ATP}), and this leads to insulin secretion. Whether SURs and K_{ATP} channels are separate molecular entities has been a subject of intense debate. Frances Ashcroft (Oxford University) presented her group's recent work showing that SUR1 does not possess intrinsic channel activity and that functional β cell K_{ATP} channels are, in fact, a complex of SUR1 and the inwardly rectifying K^+ channel $K_{IR}6.2$ (Sakura et al., 1995). SUR1 also interacts with at least three other inward rectifiers ($K_{IR}6.1$, $K_{IR}1.1$, and a channel endogenous to HEK293 cells), endowing them with sensitivity to inhibition by sulfonylureas (Ämmälä et al., 1996).

Joseph Bryan (Baylor University) argued as well that K_{ATP} channels in pancreatic β cells are aggregates of SUR1 and $K_{IR}6.2$ subunits (Inagaki et al., 1995). He offered evidence that these two proteins physically associate and suggested that complete K_{ATP} channels might form as octomers containing four of each subunit type. Bryan next reported on identification (with Alan Perlmutt's group, Washington University) of a new mutation in SUR1 that causes persistent hyperinsulinemic hypoglycemia of infancy (PHHI), an autosomal recessive disorder characterized by unregulated insulin secretion. Unlike two previously recognized mutations that result in aberrant SUR1 mRNA processing and truncate the second nucleotide binding fold (Thomas et al., 1995), the new mutation (G1479R) generates channels that are normally inhibited by ATP but are not activated by MgADP. Bryan suggested that the second nucleotide-binding fold is the site mediating MgADP activation and, thus, that SUR1 acts as the nucleotide sensor of K_{ATP} channels and ADP as the key physiological regulator in β cells (Nichols et al., 1996).

Both Bryan (with S. Seino, Chiba University) and Charles Burant (University of Chicago) reported cloning a new member of the SUR family. SUR2 has a wider tissue distribution than SUR1 including heart, adipocytes, bladder, skeletal muscle, and vascular smooth muscle but not neuronal tissue in the brain. Bryan revealed that SUR2–$K_{IR}6.2$ channels are 10-fold less sensitive to ATP and glibenclamide than SUR1 channels and are completely insensitive to diazoxide (Inagaki et al., 1996 [this issue of *Neuron*]). He further showed that SUR2 exhibits greater specificity in subunit association: it couples only with $K_{IR}6.2$ and not $K_{IR}6.1$.

Gary Grover (Bristol–Myers Squibb) reviewed his group's experience with agents that act to open myocardial K_{ATP} channels as cardioprotectants. While the molecular identity of the cardiac K_{ATP} channel remains undefined, the current is prominent in cardiac ventricular tissue. One lead compound under study (BMS 180448) reduces infarct size in selected animal models and appears to act via diazoxide-sensitive channels in cardiac mitochondria rather than on sarcolemmal K_{ATP} channels.

Heart Rate, I_{KACh}, and GIRK/CIR K^+ Channels

David Clapham (Mayo Clinic and Research Foundation) described his group's identification of the molecular components that mediate slowing of the heartbeat when acetylcholine (ACh) is secreted by the vagus nerve. He presented compelling evidence that ACh binding to atrial muscarinic receptors leads to I_{KACh} current activation in a G protein βγ subunit–dependent process. Clapham revealed that the channel underlying I_{KACh} is a heteromultimer of two distinct inwardly rectifying K^+ channel subunits, GIRK1 ($K_{IR}1.1$) and a newly cloned member of the family, CIR, which is found in the heart exclusively in the atria ($K_{IR}3.4$; Krapivinsky et al., 1995). He showed

biochemical evidence for direct binding of $G_{\beta\gamma}$ (but not G_{α}) to recombinant GIRK1/CIR channel subunits as well as to native I_{KACh} channels immunoprecipitated from cardiac tissue. These findings resolve confusion regarding three key issues. First, $G_{\beta\gamma}$ binds to and activates I_{KACh}. Second, CIR is not a component of the cardiac K_{ATP} channel; as noted above, the molecular identity of this channel is not yet known, although it seems likely to be a SUR–inward rectifier complex. Third, reflecting the heteromeric nature of the native channel in atrium, GIRK1 does not form fully functional channels unless coassembled with CIR or a CIR homolog, such as XIR, which is present in uninjected Xenopus laevis oocytes (Hedin et al., 1996).

Long QT Syndrome, I_{Kr}, and HERG

Michael Sanguinetti (University of Utah) first discussed his group's work on drugs that block I_{Kr}, the fast delayed rectifier current essential to the repolarization of the myocardium to end each heartbeat. Pharmacologic block of I_{Kr} by class III antiarrhythmic drugs can delay repolarization and induce an acquired form of long QT syndrome (LQTS). This predisposes the heart to an unusual ventricular arrhythmia called torsade de pointes, which can degenerate into ventricular fibrillation and cause sudden death. Sanguinetti also discussed a congenital form of LQTS associated with mutations in *HERG*, the gene that appears to encode the human I_{Kr} channel. Analysis of the functional consequences of *HERG* mutations reveals they cause decreased channel function, similar to the effect of drug blockade (Sanguinetti et al., 1995). Like the drug-induced arrhythmia, the congenital syndrome is characterized by torsade de pointes and can lead to sudden death. Sanguinetti reported that *HERG* (like I_{Kr} in myocytes) is activated by extracellular K^+ and that elevation of serum K^+ by $\sim$1.3 mEq/L in patients with *HERG* mutations is sufficient to normalize QT interval. Notably, among the over 20 mutations of the *HERG* gene found in humans thus far, those that diminish channel function most dramatically (for example, G628S) were isolated from patients with the most severe clinical symptoms. Three other genetic forms of LQTS have been identified involving mutations in other Na^+ and K^+ channels in the heart (LQTS, 1996).

Na^+ and Ca^{2+} Channels of the Heart and Nervous System and Channel-Blocking Drugs

William Catterall (University of Washington) discussed his group's identification of residues in Na^+ and Ca^{2+} channels that are critical for channel blockade by local anesthetics and antiarrhythmics (drugs used widely for pain reduction and treatment of abnormal patterns of cardiac electrical activity). Highlighted were point mutations in the S6 segment of the fourth internal repeat of the pore-forming α subunit of each channel. In Na^+ channels, mutations in this region reduced block by lidocaine over 30-fold, altered its use dependency, and created a new access pathway that permitted rapid blocker dissociation. Ca^{2+} channel mutations in similar locations dramatically decreased block by the phenylalkylamine (−)D888 (Ragsdale et al., 1994; Hockerman et al., 1995). That these same amino acid residues were less important in Na^+ channels for block by quinidine or phenytoin, and a distinct site in Ca^{2+} channels is responsible for block by dihydropyridines (DHPs), suggests a basis for specificity among the drugs. A model was offered to explain Ca^{2+} effects on DHP–channel interaction: a single Ca^{2+} ion within the channel may increase the apparent affinity of DHP antagonists, while two Ca^{2+} ions in the channel (representing the conducting state) makes drug binding energetically unfavorable (Peterson and Catterall, 1995).

David Triggle (State University of New York at Buffalo) discussed the widespread use of L-type Ca^{2+} channel blockers (such as nifedipine, verapamil, and diltiazem) in the treatment of hypertension, angina, cardiac arrhythmias, and peripheral vascular disease. The relevance of such consideration is revealed by a recent meta-analysis of clinical trials showing excess mortality in patients with acute myocardial infarction who received "short-acting" Ca^{2+} entry blockers like nifedipine (Furberg et al., 1995). Triggle's group is evaluating second generation DHPs now entering clinical use as well as new synthetic ligands. They find significant variation in activity, state dependence, and pharmacokinetics among the agents and suggest that differences in vascular selectivity of Ca^{2+} channel antagonists, especially the 1,4-DHPs, primarily reflects the effects of chemical structure on voltage-dependent binding (Sun and Triggle, 1995).

New Targets and Drug Candidates

Voltage-Gated K^+ Channels and the Immune System

Kv1.3 is a voltage-gated K^+ channel that has been targeted for development of new immunosuppressive agents that might have fewer toxic side effects than currently prescribed drugs. Activity of this channel is an obligatory step in T cell proliferation. Blockers of Kv1.3 are expected to display selective action because expression of the channel in peripheral tissues is highly restricted and T cells are the only cells for which Kv1.3 appears to dominate control of the membrane potential. Doug Hanson (Pfizer) discussed his group's results from a high throughput screen employing a rubidium efflux assay and cells stably overexpressing Kv1.3. A piperidine lead compound was discovered that displayed marked selectivity for Kv1.3 over other homologous K^+ channels. Studies with Kv1.3 mutants suggested that the observed selectivity was due to interactions between the benzhydryl moiety of the blocker and His-404 in the Kv1.3 outer pore. However, extensive synthetic chemistry efforts failed to increase the potency and selectivity of this class of compounds to levels suitable for further development. Efforts are currently underway to identify new chemical leads by screening a natural products library and also by pursuing a structural-based approach in collaboration with K. George Chandy's group.

Chandy (University of California, Irvine) described his group's recent success in detailing the unknown contours of the outer pore vestibule of Kv1.3 using peptide neurotoxins of known three-dimensional structure (Aiyar et al., 1995). The toxins bind in the mouth of the channel with picomolar affinity and block conduction by forming a 1:1 complex. One goal of this mapping is to identify structural attributes that make the Kv1.3 pore unique. This provides a first step toward design of compounds

that will bind to Kv1.3 with high affinity, because they are structurally complementary, but will interact poorly with the scores of related K^+ channels in the body. A histidine residue in the pore appears to provide such a singular target. James Rizzi (Amgen) presented an overview of how computer aided design was applied to the design of new Kv1.3 inhibitors. The approach combines information gleaned from studying toxin–channel interactions as well as the interaction of small molecules with the channel. No candidate compound with marked efficacy has yet surfaced in the early phase of the project.

H. Robert Guy (National Institutes of Health) used data generated by Chandy's group, as well as others studying toxin–K^+ channel interactions and the behavior of channel point mutants, to elaborate an updated short pore model of Shaker-type K^+ channels. Guy also offered a new proposal for arrangement of transmembrane helices in the channels incorporating recent information on the role of the second and fourth transmembrane segments in voltage gating (Papazian et al., 1995; Yang et al., 1996; Larsson et al., 1996). Such models have enjoyed notable success in the past (Guy and Seetharamulu, 1986). They provide direction for mechanistic experimentation and have contributed to recent enthusiasm for rational design of drugs acting on ion channels.

Ca^{2+}-Activated K^+ Channels in Vascular and Respiratory Smooth Muscle and in the Brain

The Ca^{2+}-activated K^+ (CaK) channel family is widely expressed, and compounds that modulate members of this family are expected to have utility in the treatment of stroke, epilepsy, hypertension, and asthma. Maria Garcia (Merck) discussed her group's isolation and characterization of the two subunits (α and β) that assemble to form these channels in aortic and tracheal smooth muscle. The α subunits are pore forming and can function alone; however, the β subunits are tightly associated in native tissue and increase the sensitivity of the channel to calcium over 10-fold: this leads to a shift in the voltage sensitivity of channel of over 80 mV (Knaus et al., 1994). Using a high throughput assay to identify compounds that interfere with binding of radiolabeled charybdotoxin, two lead compounds have been identified, one (a glycosylated triterpene) that favors channel opening and another (an indole diterpene) that blocks the channel. Immunolocalization reveals the presence of CaK channels in neurons with a notable predilection for presynaptic nerve terminals in rat brain sections.

Ca^{2+} Channels and Toxins from Snails and Spiders

George Miljanich (Neurex) discussed the emerging profile of the snail peptide SNX-111 (a conopeptide) as a neuroprotectant in head trauma and in the treatment of intractable pain. The compound blocks N-type Ca^{2+} channels (which are widely distributed in the brain and highly localized to the outer layers of the dorsal horn of the spinal cord). In a preclinical study, the peptide appeared to block apoptosis and reduce cerebral infarct size in animal models. In phase I/II clinical studies, the agent was administered intrathecally to 10 patients with terminal cancer and intractable pain; a clear benefit was demonstrated with no adverse effects, and the study has been expanded to about 30 patients.

Alan Mueller (NPS Pharmaceuticals) discussed his group's work with Araxin compounds, so named because the lead agents were isolated from arachnid (spider) toxins. These compounds are noncompetitive glutamate antagonists; they appear to act as open channel blockers and have pharmacological profiles that are unique. Araxin compounds have been used in rodent models and are effective as anticonvulsants, as neuroprotectants in models of ischemic stroke, and as analgesics with potency similar to morphine. NPS is pursuing the development of a clinical candidate selected from a series of small synthetic molecules ($\sim$250 kDa) that are readily produced and appear to be more potent than the original Araxin compounds.

New Biology

New Channels

Peter Agre (Johns Hopkins University) discussed the function and structure of aquaporin (AQP) water channels. This family of channels plays a fundamental role in transmembrane water movement and enjoys wide tissue distribution in humans (AQP-1 is in the kidney, eye, and capillary endothelium; AQP-2 and AQP-3 are in the kidney with the former sorted to apical and the latter to basolateral membranes; AQP-4 is in the brain in astroglial cells; AQP-5 is in the lungs). Agre indicated that insertion and turnover of AQP-2 in renal collecting duct membranes is regulated by vasopressin and that mutations in AQP-2 can cause a severe form of nephrogenic diabetes insipidus. His group employed electron crystallography and site-directed mutagenesis to reveal the tetrameric organization of AQP-1 and its unique obverse internal symmetry (Walz et al., 1995).

Alok Mitra (Scripps Research Institute) took the audience through the process of performing electron crystallography using frozen-hydrated two-dimensional crystals of AQP-1 from human erythrocytes (Mitra et al., 1995). He showed that each AQP-1 monomer has a separate vestibule leading to a water-filled cavity enclosed by multiple transmembrane α helices.

Richard Lewis (Stanford University) discussed his group's work on the function of Ca^{2+} release–activated Ca^{2+} (CRAC) channels present in T cells. Through study of mutant T cell lines, they revealed CRAC channels to be the only Ca^{2+} influx pathway activated by T cell antigen receptors. Moreover, this Ca^{2+} influx was found to be essential for triggering the genes associated with immune activation, such as interleukin-2 (IL-2). Modulators of CRAC channels thus offer a new target for immunosuppressive therapy. Lewis indicated that depletion of internal Ca^{2+} stores acts to open CRAC channels, while increased intracellular Ca^{2+} levels inhibits their activity. This leads to oscillations in internal T cell Ca^{2+} levels. Oscillations were most prominent at low levels of antigen stimulation and, surprisingly, were more potent than matched constant elevations of Ca^{2+} for inducing expression of IL-2 reporter constructs (Zweifach and Lewis, 1995).

Alan North (Glaxo Institute) described his group's recent work on P2X receptors, the cation-selective ion channels that open on binding of extracellular ATP. mRNA for P2X channels is found widely through the

brain and spinal cord where the channels appear to play a role in fast synaptic transmission between neurons and in communication between autonomic nerves and smooth muscles. P2X mRNA is also expressed in T cells and pituitary gland tissue, suggesting a possible role for these channels in immune and endocrine function (Lewis et al., 1995). The six members of the family cloned thus far are 36%–48% identical and show no relation to other ligand-gated ion channels. The six clones show differences in tissue distribution as well as diversity in gating, agonist sensitivity, and blocker sensitivity. They all appear to have two membrane-spanning segments with a large extracellular loop.

Bernard Rossier (Université de Lausanne) discussed amiloride-sensitive epithelial Na^+ channels (ENaCs). These channels are critical for water and salt homeostasis and are expressed in the kidney, colon, and lung. ENaCs assemble as heteromultimers of three homologous subunits (α, β, and γ) with unknown stoichiometry. Mutations that result in increased ENaC activity lead to hypertension. Liddle's syndrome is an autosomal dominant disease characterized by early onset hypertension due to constitutive ENaC activity and salt accumulation; two mutations that cause the syndrome truncate of the C-terminal region of the β (Schild et al., 1995) or γ subunit (Hansson et al., 1995). Conversely, mutations that decrease ENaC activity appear to lead to salt wasting and respiratory distress. Thus, homozygous transgenic mice in which ENaC genes have been knocked out developed acute respiratory distress and cyanosis postpartum. The animals died within 48 hr of birth due to excess fluid in the lungs; this suggests a critical role for ENaCs in normal fluid clearance from the lungs.

Channel Diversity

John Adelman (Vollum Institute), whose earlier studies highlighted the wide variety of CaK channels (over 100) that results from alternative splicing (Lagrutta et al., 1994), offered yet another dimension to the structural complexity of this channel family. Using a variant of the yeast two-hybrid system, his laboratory has identified six new genes whose protein products associate with the C-terminal domain of a Drosophila CaK channel. One clone appears to yield a soluble protein, is expressed in the developing nervous system of flies, and acts to diminish CaK currents when coexpressed in Xenopus oocytes. The effect is specifically reversed when the C-terminal portion of CaK is also expressed, suggesting the new clone is an additional modulatory subunit of CaK channels.

Michael Harpold (SIBIA) discussed his group's efforts to screen for subtype-selective modulators of neuronal Ca^{2+} channels. He revealed this goal to be complicated by the existence of wide combinatorial diversity in Ca^{2+} channel subtypes. These channels are composed of three distinct subunits (α_1, $\alpha_2\delta$, and β). At least five genes encoding different pore-forming α_1 subunits and four genes encoding different β subunits have thus far been identified in the nervous system. Moreover, splice variants of α_1 and β subunits have been identified that form channels with unique gating and conduction attributes when coexpressed with a neuron-specific $\alpha_2\delta$ subunit. Bravely, cell lines expressing various human subunit combinations have been established for evaluation using an automated Ca^{2+} fluorescence-based screen with a capacity of >600 assays each hour.

RNA Editing, Glutamate Receptor Channels, and the Central Nervous System

Glutamate receptors (GluRs) are ligand-gated channels that conduct Ca^{2+} and monovalent cations. These channels mediate fast excitatory neurotransmission in the central nervous system of humans and are speculated to play an important role in learning as molecular coincidence detectors. GluRs exhibit wide molecular diversity as a result of multiple GluR genes, alternative transcript splicing, and combinatorial variation. Steve Heinemann (Salk Institute) and Peter Seeburg (ZMBH, Heidelberg) discussed their groups' efforts to understand the basis of yet another type of GluR diversity. The Ca^{2+} permeability and rectifying properties of these channels are subject to regulation by a change in the amino acid present at a unique channel position (Hume et al., 1991). Underlying this change is an RNA editing process involving deamination of adenosine to inosine that was first elucidated in two kainate-sensitive GluR subunits (Sommer et al., 1991). Thus, while the gene for GluR2 encodes a glutamine (CAG) at this position, editing yields a protein with an arginine (CI/GG) at the site. RNA editing is a nuclear process that targets specific adenosines and requires a double-stranded RNA structure configured from complementary exonic and intronic sequences (Higuchi et al., 1993). The editing mechanism is specific—only GluR2 RNA is edited in a glial cell line even though GluR1, GluR3, GluR4, and GluR6 have similar sequences at the site—and depends on an intronic sequence in the GluR2 gene that is sufficient to cause editing of GluR3 in a chimeric construct (Egebjerg et al., 1994). The occurrence of inosine in other mammalian mRNAs is expected since HeLa cell extracts can edit GluR pre-mRNA with positional accuracy (Melcher et al., 1995).

Heinemann also discussed the role of the extracellular S2 channel segment in ligand affinity showing that it determines the kainate affinity of GluR chimeras into which it is grafted (Stern-Bach et al., 1994). Seeburg's group found that the C-terminal cytoplasmic domain of N-methyl-D-aspartate (NMDA) receptors could interact with postsynaptic density protein PSD-95 in a yeast two-hybrid assay. This is consistent with their observation of similar expression patterns for PSD-95 and NMDA receptor transcripts in rat brain as well as their colocalization in cultured rat hippocampal neurons. Seeburg suggested that interaction of these proteins may affect the plasticity of excitatory synapses (Kornau et al., 1995).

Channel Structure and Function

Richard Aldrich (Stanford University) offered data to support the novel proposition that inward rectification and outward rectification in K^+ channels can result solely from a shift in the voltage dependence of channel activation. Thus, wild-type Shaker channels pass outward K^+ currents when the membrane is depolarized from a negative resting potential of -80 mV to 0 mV; the channels undergo a conformational change from resting to open state with a midpoint for activation of -40 mV. If the membrane remains depolarized (0 mV), channels rapidly move to a nonconducting inactivated state that results from block of the open channel by its cytoplasmic

N-terminus. On the other hand, Shaker channels whose midpoint for activation is shifted below −200 mV by point mutation are chronically inactivated at a resting potential of −80 mV. These channels show no outward currents on depolarization (they are already opened and inactivated) but show inward currents on further hyperpolarization as they recover from inactivation and move through the open channel state on the way to the resting closed state (Miller and Aldrich, 1996).

Kevin Campbell (University of Iowa) reported his group's examination of the molecular basis for subunit interactions in voltage-gated Ca^{2+} channels using a direct binding assay. Mutations in the α_1 subunit that ablate binding to an in vitro translated β subunit suggest that the α_1–β interaction involves the cytoplasmic loop between homologous domain I and II of the α_1 component. The α_1 subunit was found to interact with various β subtypes from native tissues and a single tyrosine residue in α_1 was found to be critical for this interaction (Witcher et al., 1995; De Waard et al., 1995). Interestingly, the δ subunit is critical for the α_1–$\alpha_2\delta$ interaction and the eighteen potential glycosylation sites in the $\alpha_2\delta$ subunit appear to stabilize the interaction (Gurnett et al., 1996).

Steve Goldstein (Yale University) discussed his group's studies of minK, a protein with just 130 amino acids that is found in cardiac, renal, uterine, auditory, and gastrointestinal tissue. MinK bears no homology to known channels, but its expression in Xenopus oocytes yields K^+ currents remarkably similar to those underlying cardiac repolarization and found in auditory epithelia. Goldstein presented data supporting the direct contribution of minK residues to the lining of a K^+ channel pore. He argued that functional channel complexes contain just two minK monomers and that an as yet unidentifed non-minK protein appears to be required for channel function and may contribute to the pore as well (Wang and Goldstein, 1995; Wang et al., 1996).

Steve Siegelbaum (Columbia University) offered new evidence about the structural basis for opening of cyclic nucleotide–gated (CNG) cation channels from retinal and olfactory epithelia, building on his group's previous progress (Goulding et al., 1994). He showed that activation of these channels is determined by two channel domains: first, a 25 residue domain in the C-terminal region that participates in ligand binding and thus determines selectivity between cAMP and cGMP (this region is homologous to the C-helix in the Escherichia coli protein CAP). The second domain is a large N-terminal region that determines differences in gating between channel isoforms. To understand how this non-ligand-binding domain of the channel might exert this influence, they studied spontaneous openings of channel chimeras. Siegelbaum argued convincingly for an allosteric model in which the N-terminal domain of the protein influences channel open probability because it determines the free energy difference between closed and open states of the channel in the absence of ligand.

Ca^{2+} Mediation of Vascular Tone, Immunity, and Pancreatic Function

Mark Nelson (University of Vermont) discussed how control of cerebrovascular tone by smooth muscle involves small local releases of Ca^{2+} from sarcoplasmic reticulum through ryanodine-sensitive Ca^{2+}-release channels. These Ca^{2+} "sparks" (so-called because of their appearance when visualized with Ca^{2+} sensitive dyes) were observed just under the surface membrane of single smooth muscle cells from myogenic cerebral arteries. Ca^{2+} sparks appear to result from opening of only 1 or 2 channels that, in turn, activate 20–100 CaK channels leading to cellular hyperpolarization and arterial dilation. These effects on membrane permeability were manifest electrophysiologically as spontaneous transient outward currents (STOCs). Ca^{2+} sparks have little effect on spatially averaged Ca^{2+} levels that regulate arterial contraction (Nelson et al., 1995).

Michael Cahalan (University of California, Irvine) discussed the emerging roles of K^+ and Ca^{2+} channels in lymphocyte function (Negulescu et al., 1994; Lewis and Cahalan, 1995). T cell activation begins with stimulation of T cell antigen receptors, activation of Kv1.3 K^+ channels, and influx of Ca^{2+} through CRAC channels. (The K^+ channels support a negative membrane potential that drives Ca^{2+} influx.) Within seconds, a sustained rise in intracellular Ca^{2+} levels occurs leading to IL-2 gene expression; this cytokine is essential for T cell proliferation and immune response. As noted above, blockade of Kv1.3 inhibits antigen-driven activation of lymphocytes, probably by diminishing Ca^{2+} oscillations that result from activation of plasma membrane CRAC channels. Using video-imaging techniques, Cahalan's group tracked Ca^{2+} signals in individual T cells and measured expression of a reporter gene controlled by the NF-AT element of the IL-2 enhancer. Raising Ca^{2+} from resting levels increased the fraction of cells showing gene expression and decreased mobility of the cells (presumably this fosters T cell accumulation at sites of stimulatory antigen). Ca^{2+}-dependent IL-2 gene expression was enhanced by activation of protein kinase C but inhibited by stimulation of protein kinase A.

Iain Dukes (Glaxo Institute) presented his group's efforts to understand the molecular basis for glucose stimulated secretion of insulin by β cells of the pancreas (Worley et al., 1994; Philipson et al., 1994). Insulin secretion is associated with β cell membrane depolarization and elevation of intracellular Ca^{2+} levels. Dukes showed that depleting intracellular Ca^{2+} stores induced membrane depolarization and led to a sustained Ca^{2+} influx; on the other hand, interventions promoting Ca^{2+} store refilling produced hyperpolarization and inhibited Ca^{2+} influx. These results suggest a role for nonselective cation channels that are stimulated by depletion of intracellular Ca^{2+} stores. They examined the role of voltage-gated K^+ channels in insulin secretion by producing a transgenic mouse strain that overexpresses an insulinoma-derived K^+ channel in its pancreatic β cells. Whole-cell recordings confirmed expression of the channels in transgenic islet cells; expression was correlated with hyperglycemia and hypoinsulinemia and supports a role for K^+ channels in excitation–secretion coupling.

New Methods

Roger Tsien (University of California, San Diego) described the advances his group has made using fluorescence resonance energy transfer to assess rapid changes in transmembrane voltage in individual cells as well as to study protein–protein interactions and gene

expression. Previously, voltage-sensing dyes were either sensitive and slow or insensitive and fast. A two component system now allows both sensitivity and speed (time constant ≈ 2 ms). The approach employs, first, a hydrophobic fluorescent anion that moves rapidly from one face of the membrane to the other in response to voltage changes; the second fluorophore is fixed to the extracellular surface and undergoes energy transfer with the membrane bound probe. High speed confocal microscopy was achieved as was continuous monitoring of beating cardiac myocytes through elimination of movement artifact by ratiometric measurements (Gonzalez and Tsien, 1995). Tsien also described a new reporter gene system utilizing β-lactamase to separate donor from acceptor fluorophores; the method is sensitive to as few as 10–100 molecules of β-lactamase per cell and allows visualization of gene expression in individual viable cells (Hein and Tsien, 1996).

Vincent Groppi (Upjohn Pharmaceuticals) described his group's experience with FLIPR, an automated system for measuring changes in membrane potential as a means of rapidly screening K^+ channel modulators. In a screen for potential K^+-sparing diuretics, they are achieving a capacity of 20,000 compounds every month (by one person!) using cells stably expressing ROMK1, a renal inward rectifier K^+ channel. Thus far, 0.5% of the compounds show activity by both FLIPR and a secondary pharmacological assay and ~10% of these show activity in laboratory animals.

Conclusion

The vast array of medical conditions potentially amenable to treatment by drugs that act on ion channels makes the pursuit of these investigations attractive and compelling. However, the best route to designing safe and effective agents remains uncertain. From a practical standpoint, patients (and corporate managers) want to know "will all this new technology and mechanistic insight shorten the discovery process? Will it give me an effective and safe drug before I become sicker? Will it reduce development costs by increasing the likelihood of clinical success?" Some symposium participants felt ion channels to be a poor choice for rational drug development on first principles. Channels, they correctly emphasized, are moving targets. While almost all screening assays evaluate drug binding to static channel states, therapeutic efficacy reflects dynamic drug interactions with systems that respond to voltage, time, stimulation patterns, and local environment. In addition, molecular assignment of native currents to the cloned channels used for screening has proven an elusive goal and, as a result, the relevance of assays to in vivo function is questionable, especially if cells vary the subunit composition of their channels.

Other symposium participants were more upbeat; they thought our field merely too young to be the subject of rational development strategies, but one that held considerable promise. Too much basic biology is still emerging, they argued, the problems ahead have not yet been adequately defined. As evidence, they pointed to the dizzying diversity of newly recognized channel families and subtypes, modulatory accessory subunits, combinatorial possibilities for channel assembly, and secondary mediator pathways that had been presented at this symposium alone. The optimists among us simply saw the cacophony of biological, functional, and structural complexity as new opportunity for specificity in drug action. All conferees agreed, however, that the symposium had produced an amalgamation and of old and new ideas as well as a confederation of academic and industrial scientists that heralded the maturing of the endeavour.

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