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Ion channels in health and disease

83rd Boehringer Ingelheim Fonds International Titisee Conference

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Introduction

Ion channels are key molecules for signal transduction across biological membranes. Combining physiological experiments with DNA sequence data has recently linked many diseases to defects in ion channels and, to acknowledge this, the term 'channelopathies' has been coined. While drugs acting on ion channels have long been used as therapeutics, their potential for specific treatment of channelopathies is only now beginning to be elucidated. Plasma membrane ion channels are easy to access and are often expressed at relatively low concentrations in specified cells and tissues, which can make them excellent targets for drug design. While new ion channels are still being characterized, an important dimension has been added to ion channel research: understanding their relevance in pathophysiological processes and ion channel linked diseases. To acknowledge this development, the Boehringer Ingelheim Fonds (http://www.bifonds.de), an independent foundation for basic research in medicine, has focussed its 83rd International Titisee Conference on 'Ion channels in health and disease'. About 50 researchers from Europe, USA and Japan met in Titisee, Germany from March 21–25, 2001 to learn from each other how different ion channels are involved in physiological and pathophysiological cell functions. The discussed channel defects and the correlated diseases are summarized and referenced in Table I. In the following report, ion channels are grouped according to their selectivity and their activation mechanism.

Voltage-operated potassium channels

K^+ channels are expressed in most cell types, indicating their vital role for cell signalling. They are probably the largest ion channel family allowing for a great diversity of expression pattern in different tissues. Their best known role is that of regulating the cell's membrane potential, which, in turn, is a key regulator of many cellular processes, some of which will be outlined below.

In her keynote lecture, Frances Ashcroft (Oxford, UK) introduced K_{ATP} channels. She showed that these channels are regulated by intracellular adenosine nucleotides, and are important for physiological processes such as insulin secretion, regulation of vascular smooth muscle tone and the response to cardiac or cerebral ischaemia. Defective regulation may result in congenital hyperinsulinism and some rare forms of diabetes. The K_{ATP} channel is a heteromultimer consisting of inward rectifier Kir6.1 or Kir6.2 subunits together with sulfonylurea receptor SUR (SUR1, SUR2A or SUR2B) subunit. Ashcroft showed that K_{ATP} channel function appears to be tissue dependent and can be blocked differentially by sulfonylureas like gliclazide or glibenclamide: gliclazide blocks only K_{ATP} channels containing SUR1, whereas glibenclamide blocks all types. The efficacy of K_{ATP} channel openers may also vary with the SUR isoform. Thus, drugs that differentiate between different types of K_{ATP} channels might become useful in fighting diseases related to K_{ATP} channel malfunction.

Big potassium (BK) channels function as negative feedback regulators of vascular tone, linking membrane depolarization and Ca^{2+} sparks to depolarizing spontaneous transient outward K^+ currents (STOCs). Auxiliary BKβ1 subunits increase the sensitivity of the BKα subunit to changes in membrane potential and to internal calcium concentration ($[Ca^{2+}]_i$). To assess the *in vivo* functions of β1, Olaf Pongs (Hamburg, Germany) and co-workers constructed a knock-out (KO) mouse lacking the *slo* gene. The β1 subunit encoded by this gene is normally present in smooth muscle but not in the CNS. Analysis of the β1 KO mice revealed: (i) uncoupling of sparks and STOCs, (ii) increased constriction in cerebral arteries and, (iii) hypertension and cardiac hypertrophy. Surprisingly, acute blood pressure regulation, which is dependent on smooth muscle contraction, appeared to be normal. Thus, BK-channel dysfunction in mouse smooth

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Table I. Diseases that were discussed at the meeting and have a known ion channel link

Channel type	Gene	Channelopathy and phenotype	Reference
K^+ channel	*KCNA1*	episodic ataxia type 1 (EA-1)	Browne *et al.* (1994)
	KCNH1 (hEAG)	oncogenic potential	Pardo *et al.* (1999)
	KCNJ11	persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI)	Inagaki *et al.* (1995)
	KCNQ2/3	benign familial neonatal convulsions (BFNC)	Singh *et al.* (1998) Charlier *et al.* (1998)
	KCNQ4	hereditary hearing loss (DFNA2)	Kubisch *et al.* (1999)
	SUR1	persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI)	Thomas *et al.* (1995)
Na^+ channel	*SCNN1(ENaC)*	Liddle's syndrome (heriditary hypertension, pseudohypoaldosteronism type 1)	Chang *et al.* (1996)
	SCN4A	hyperkalaemic periodic paralysis, hypokalaemic periodic paralysis, paramytonia congenita	Fontaine *et al.* (1990) Bulman *et al.* (1999) Ptacek *et al.* (1992)
	SCN1B	generalized epilepsy with febrile seizures type 1	Wallace *et al.* (1998)
Ca^{2+} channel	*CACNA1A*	episodic ataxia type 2, familial hemiplegic	Ophoff *et al.* (1996)
	CACNA1	migraine, spinocerebellar ataxia type 6	Zhuchenko *et al.* (1997)
	SRYR1	hypokalaemic periodic paralysis	Ptacek *et al.* (1994)
	CAT-L	malignant hyperthermia, central core disease	Quane *et al.* (1993) Zhang *et al.* (1993)
	CAT-L	expressed in advanced prostate cancer	Wissenbach *et al.* (2001)
Glycine receptor	*GLRA1*	hyperplexia	Shiang *et al.* (1993)
Acetylcholine receptor	*CHRNA1*	congenital myasthenia	Sine *et al.* (1995)
	CHRNA4	autosomal dominant nocturnal frontal lobe epilepsy	Steinlein *et al.* (1995)
CNG channels	*CNGA3*	achromatopsia-2	Kohl *et al.* (1998)
	CNGB3	achromatopsia-3	Kohl *et al.* (2000)
Cl^- channel	*CLCN1*	myotonia congenita (dominant or recessive)	Koch *et al.* (1992)
	CLCN5	Dent's disease (proteinuria and hypercalciuria)	Fisher *et al.* (1994)
	CLCN7	osteopetrosis	Kornak *et al.* (2001)

References are to publications that make the initial link. Further information can be found at: http://www.neuro.wustl.edu/neuromuscular/mother/chan.html and http://www.ncbi.nlm.nih.gov/omim/ or in *Ion Channels and Disease* (2000) by F.M. Ashcroft, Academic Press or *Channelopathies* (2000) by F. Lehmann-Horn and K. Jurkat-Rott (eds), Elsevier.

muscle cells alters the vasoregulation of systemic blood pressure and causes hypertension.

Éther-a-gó-gó (EAG) K^+ channels, originally identified based on the phenotype of abnormal leg shaking under ether anaesthesia in *Drosophila* mutants, are normally expressed only in the CNS, with little or no expression of mRNA or protein detected in other tissues. L. Pardo (Göttingen, Germany) presented evidence that there is a reason for this carefully regulated expression; ectopic expression of these channels has oncogenic potential. Cell lines of cancerous origin, as well as tissue originating from patients affected by cancer, express high levels of EAG, and the injection of cells transfected with EAG induces solid tumours within two weeks in immunodeficient (SCID) mice. Furthermore, inhibition of EAG channel expression by antisense oligodeoxynucleotides inhibits tumour cell proliferation, indicating a causative role of EAG in cancer. K^+ and Na^+ channels also have a role in regulating muscle relaxation, as was discussed by F. Lehmann-Horn (Ulm, Germany). He gave a general overview of the various forms of periodic paralyses that involve a variety of ion channels as summarized in Table I.

Non voltage-operated sodium channels

The best-known Na^+ channels are those responsible for action potentials and are activated by voltage. There are, however, a variety of Na^+ channels that are not activated by voltage but rather modulated by a number of hormones and messengers. One of those, the epithelial Na^+ channel (ENaC) is responsible for Na^+ re-uptake across the apical membrane of distinct epithelial cells in the kidney. ENaC is a member of the DEG/ENaC superfamily. While Michael J. Welsh (Iowa City, IA) focussed on the degenerin (DEG) side of the family, Bernard C. Rossier (Lausanne, Switzerland) presented data linking ENaC to human diseases. Liddle's syndrome is characterized by an early onset of a severe hypertension caused by increased Na^+ re-uptake through hyperactivity of ENaC. Mutations in either the β or γ subunit of ENaC increase its activity through an increase in channel number or open probability, respectively. The increased channel number is most likely due to reduced channel internalization because the Liddle's mutations alter the binding of ENaC to the Nedd4 protein, which is important for ENaC internalization. Rossier also discussed how decreased ENaC activity leads to pseudohypoaldosteronism type 1, a rare inherited disorder in which the kidney does not respond to aldosterone. He introduced mouse models with complete and partial KOs of ENaC, which should prove helpful for studying the disease in detail. Welsh switched to the degenerins of *Caenorhabditis elegans*, which are proteins for which gain-of-function mutants can cause cell swelling, vacuolation and eventually cell death. He presented data on members of this family that are involved in sensory transduction. These are expressed in nerve endings of

specialized sensory structures in the skin and tongue and respond to a wide variety of sensory stimuli, i.e. salt taste, touch and mechanosensation, and protons. One of these channels whose activity is increased by low temperature might underlie the so-called Weber phenomenon, a century-old observation that a cold iron ball appears to be heavier than a warm one.

Voltage-operated calcium channels

Voltage-operated Ca^{2+} channels have long been recognized as drug targets for various illnesses ranging from migraine to heart disease. Understanding their function in health and disease was the focus of a number of presentations: Jörg Striessnig (Innsbruck, Austria) summarized the various channelopathies resulting from mutations in voltage-activated Ca^{2+} channel genes including heart failure, ischaemia, Lambert-Eaton myasthenia, colon inflammation, epilepsy or migraine. He then introduced the generation and features of mouse models that harbour mutations in the dihydropyridine (DHP)-sensitive L-type Ca^{2+} channels of the α1D ($Ca_v1.3$) and the α1C ($Ca_v1.2$) types. Inactivation of the *$Ca_v1.3$* gene yields viable but deaf mice that show cardiac bradycardia and arrhythmia. These findings indicate that α1D type Ca^{2+} channels are essential for normal auditory function and may also control cardiac pacemaker activity. Currently available DHPs modulate both α1C and α1D L-type Ca^{2+} channels. The generation of DHP-resistant $\alpha1C^{DHP-}$ mice, as reported by Striessnig, appears to be a promising model for predicting the pharmacotherapeutic potential of DHP-type modulators selective for α1D channels.

To finally understand epilepsy and migraine, it is of great importance to study neuronal P/Q type Ca^{2+} channels. William Catterall (Seattle, WA) presented results on the interactions of those channels (in particular Ca_v 2.1) with the synaptic vesicle proteins syntaxin, SNAP-25 and synaptotagmin. He showed that the interactions are isoform specific, Ca^{2+}-dependent, reduced by phosphorylation and needed for efficient synchronous transmitter release. The interaction with SNARE proteins is important for retrograde modulation of Ca^{2+} channel inactivation. He went on to dissect the role of β subunits and Ca^{2+}/calmodulin in the facilitation and inactivation of α_{1A}-mediated Ca^{2+} currents and identified a novel Ca^{2+} binding protein (CaBP-1) that accelerates inactivation independently of Ca^{2+}-influx. CaBP-1 competes with calmodulin for channel binding and can act as a switch between Ca^{2+}- and voltage-dependent inactivation. A detailed analysis of the binding site and function of calmodulin binding in inactivation and facilitation has been published previously and was presented by Harald Reuter (Bern, Switzerland). Whether migraine pain is caused by a defect in Ca^{2+} channel function is not known at present. However, the role of the same Ca^{2+} channels in other types of pain is better understood. Tsutomu Tanabe (Tokyo, Japan) discussed the roles of Ca_V 2.1, Ca_V 2.2 and Ca_V 2.3 in pain. In each case, he compared pain-related behaviours of wild-type to that of KO mice. While Ca_V 2.1 KO mice showed impairment in the sensation of acute somatosensory pain, Ca_V 2.2 KO mice showed decreased fear in the elevated maze test and reduced pain responses that correlate with a suppression of neuropathic pain. Ca_V 2.3 KO showed increased anxiety and abnormal nociceptive and antinociceptive behaviours, suggesting multiple roles for $Ca_v2.3$ in the nervous system.

Ca^{2+} channels not only couple to the secretion machinery in neuronal cells but also in many other cell types. Per-Olof Berggren (Stockholm, Sweden) focussed on the functional impact of Ca^{2+} signalling and Ca^{2+} entry in pancreatic β-cells. He showed that syntaxin 1 colocalizes and associates with the α1D subunit of the voltage-gated L-type Ca^{2+} channel and thereby modulates Ca^{2+} channel activity and insulin release. He discussed that serum from patients with newly diagnosed type 1 diabetes activates L-type Ca^{2+} channels, leading to increased $[Ca^{2+}]$ and apoptosis, and that these effects can be prevented by Ca^{2+} channel blockers. In mice deficient in the β3 subunit of L-type Ca^{2+} channels, glucose-induced oscillations in $[Ca^{2+}]$ and insulin secretion are increased, indicating that the β3 subunit negatively modulates Ca^{2+} signalling and insulin exocytosis.

Another fundamental role for voltage-operated Ca^{2+} channels is the one in muscle excitation-contraction coupling. Kurt Beam (Fort Collins, CO) focussed on the coupling between the α_{1S} ($Ca_v1.1$) Ca^{2+} channel and the ryanodine receptor (RyR) in skeletal muscle. While both channel and receptor are needed for survival, it still is not clear how they interact. Beam's group succeeded in defining the critical domain of α_{1S} and he showed that a 45 amino acid-residue region might be the interaction site required for bidirectional coupling between α_{1S} and the RyR. A number of mutations in humans have been shown to cause hyperactivity of RyR1 and to result in malignant hyperthermia and/or central core disease. More recently, it has been shown that a mutation causing hypo-activity of RyR1 can also result in central core disease, a human congenital myopathy characterized by fetal hypotonia and proximal muscle weakness.

Store- and receptor-operated calcium/cation channels

Voltage-operated Ca^{2+} channels are usually not present in electrically non-excitable cells. However, those cells possess another type of Ca^{2+} channel. Richard Lewis (Stanford, CA) introduced store-operated Ca^{2+} channels (SOCs), which, upon depletion of internal Ca^{2+} stores, meditate Ca^{2+} influx from the extracellular space. This so-called store-operated Ca^{2+} entry mechanism supports cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) elevations during receptor stimulation. Its physiological importance is apparent in certain forms of immunodeficiencies associated with the absence of antigen-triggered Ca^{2+} influx and defective T cell proliferation. The sequence of events leading to the opening of SOCs is far from clear. Multiple signalling mechanisms have been postulated, including direct interaction between the inositol triphosphate receptor ($InsP_3R$) within the endoplasmic reticulum and the SOCs in the plasma membrane. In favour of this model is the evidence that 2-aminoethoxydiphenyl borate (2-ABP), a membrane permeant $InsP_3R$ antagonist, also prevents store-operated Ca^{2+} entry. However, Lewis presented crucial arguments against the involvement of $InsP_3R$ in SOC activation in a B lymphocyte cell line: removal of all three $InsP_3R$ isoforms in these cells by genetic means has no effect on SOCs activity. Furthermore, in the triple KO cell line, 2-APB was still able to block Ca^{2+} influx, suggesting that its effect on SOCs is independent of IP_3R function. Lewis went on to point out that

2-APB acts on SOC channels in a direct but complex manner. Depending on the concentration of 2-APB used in the experiments it could either inhibit or facilitate SOC activity in lymphocytes. The molecular nature of SOCs is still not known. The best candidate genes are those from the so-called transient receptor potential (TRP) family. The TRP protein, which mediates light-activated Ca^{2+} entry in *Drosophila* photoreceptors, has been considered a paradigm for SOCs. However, the events leading to TRP gating, downstream of phospholipase C (PLC) activation, remain unclear. Roger Hardie (Cambridge, UK) presented data indicating that diacylglycerol (DAG) or its metabolites, but not inositol 1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+} release, is required for photoreceptor excitation. Indeed, phototransduction was normal in *Drosophila* photoreceptors lacking the *$InsP_3R$* gene, whereas TRP channels were constitutively active in diacylglycerol kinase (DGK)-deficient flies. Hardie also showed that Ca^{2+} entry through TRP channels regulates phosphatidylinositol 4,5-bisphosphate (PIP_2) recycling and that the inability of TRP mutants to quickly recover after prolonged light stimulation may be due to PIP_2 exhaustion. Several gene products that have been cloned from vertebrates share moderate to high sequence identity with *Drosophila's* TRP channel. On the basis of sequence and structural homology, the TRP family can be divided into three groups: short TRPs (STRPs), long TRPs (LTRPs) and osmoregulated TRPs (OTRPs). While STRPs appear to trigger a range of receptor-mediated Ca^{2+} influx phenomena, little is known about the function of LTRPs. Members of the OTRPs can confer osmosensitivity and are involved in the pain pathway. Tim Plant (Berlin, Germany) presented data demonstrating that OTRPC4, which shares sequence identity to OSM-9, a putative TRP-related channel protein from *C. elegans*, forms Ca^{2+} permeable non-selective cation channels. These channels display spontaneous activity in isotonic media and are activated by a decrease in extracellular osmolarity and inhibited by an increase. OTRPC4 (syn. TRP12 or VR-OAC) appears to be expressed in the epithelium of the distal convoluted tubule of the mouse kidney and may represent a candidate for an osmosensor in cellular volume regulation, not only in kidney but also in other cells of the body.

TRP4 belongs to the STRP family and is thought to be involved in store-operated Ca^{2+} entry. TRP4 KO mice have recently been generated by Marc Freichel (Homburg, Germany) who presented data supporting the involvement of TRP4 in store-operated Ca^{2+} entry of endothelial cells and in the regulation of blood vessel tone. He showed that store-operated Ca^{2+} currents in cultured aortic endothelial cells from KO mice were significantly reduced compared to wild-type tissue resulting in decreased Ca^{2+} signalling and impaired vasorelaxation of aortic rings. David Clapham (Boston, MA) showed that TRP5 (and also TRP4) is able to associate with TRP1 to form heteromultimeric channels with distinct properties from the homomeric channels. TRP5 and TRP1 can be co-immunoprecipitated with the PLCβ from mouse brain, suggesting that structures analogous to the *Drosophila* transducisome protein complex may exist in mammalian cells. Clapham also described properties of the recently identified TRP-PLIK protein, which belongs to the LTRP subgroup. TRP-PLIK is both an ion channel and a protein kinase, and the kinase activity appears to be essential for the channel function.

Bernd Nilius (Leuven, Belgium) and Ulrich Wissenbach (Homburg, Germany) discussed properties of two other members of the OTRP group. Both proteins, the epithelial Ca^{2+} channel ECaC (syn. ECaC1) and the closely related Ca^{2+} transport protein CAT1 (syn. ECaC2), form very selective Ca^{2+} channels when heterologously expressed. While Nilius focussed on comparing the electrophysiological properties of CAT-1 and ECaC concerning selectivity, inactivation and pore properties, Wissenbach introduced CaT-Like, a channel almost identical to human CaT1, which is highly expressed in advanced prostate cancer but is not detectable in healthy prostate tissue and benign prostatic hyperplasia. Thus, this protein could potentially be used as a marker for prostate cancer progression as well as being a new target for chemotherapy. Both CAT-1 and CAT-Like form constitutively activated channels when expressed in HEK293 or CHO cells. Given the importance of receptor- and store-operated Ca^{2+} entry for cell proliferation and activation, it appears likely that the underlying ion channel proteins are prime candidates to play a role in as yet poorly understood diseases (i.e. cancer or diseases of the immune system).

The $InsP_3$ receptor is thought to be a key player in receptor- and store-operated Ca^{2+} entry and Katsuhiko Mikoshiba (Tokyo, Japan) and Franz Hofmann (München, Germany) focussed on $InsP_3$-dependent signalling processes. Active $InsP_3$-Ca^{2+} signalling via the $InsP_3$Rs functions as a ventral signal in patterning of the body axis during early embryonic development in *Xenopus*. In hippocampal CA1 neurons of $InsP_3R1^{-/-}$ mice, long-term potentiation (LTP) is facilitated, indicating that the $InsP_3R1$ is involved in the suppression of LTP in wild-type cells. Chemical compounds like 2-aminoethoxydiphenyl borate and derivatives, which interfere with $InsP_3$-Ca^{2+} signalling, might be important tools to further dissect the links between Ca^{2+} depletion of $InsP_3$-sensitive stores and Ca^{2+} entry into cells. Hofmann continued on the regulation of Ca^{2+} release from $InsP_3$-sensitive stores by a signalling complex that includes the $InsP_3$R-associated cGMP kinase substrate (IRAG), the $InsP_3$R and the cGMP dependent protein kinase type Iβ. CyclicGMP kinase is a major target of the nitric oxide/cGMP pathway and, at least in smooth muscle cells, phosphorylation of IRAG by cGMP kinase is a major mechanism that inhibits $InsP_3$-induced Ca^{2+} release, and thereby reduces intracellular Ca^{2+} and relaxes vascular tone.

Ligand-operated cation channels

In addition to having other roles, cation channels activated by ligands (i.e. neurotransmitters or hormones) are important for synaptic transmission. Heinrich Betz (Frankfurt, Germany) introduced the structural basis of ligand recognition by glycine- and NMDA receptors, and Cord-Michael Becker (Erlangen, Germany) focussed on the pathological potential of glycine receptor dysfunction. Glycine receptors are composed of an α3β2 complex, where α is the ligand-binding subunit and the β subunit is required for proper localization of the channel protein to the cell surface. The latter is accomplished by the participation of the β subunit in a supramolecular complex, one of whose components, Gephyrin, binds to the channel and prevents its endocytic removal from the cell surface. The mapping of the glycine binding site identified a FXY domain (where X is a small amino acid) to be important for agonist binding. Mutant alleles

of glycine receptor α- and β-subunit genes underlie hypertonic motor disorders in humans and mice, e.g. hyperekplexia (startle disease, stiff baby syndrome). Causes of hyperekplexia in humans include recessive glycine receptor α1 (*GLRA1*) mutations that either affect assembly and sorting of the receptor or lead to a complete loss of function, and also the dominant P250T *GLRA1* allele, for which the receptor is expressed but shows altered properties. Several murine models offer the possibility of detailed analysis of the effects of glycinergic dysfunction. For example, in the mutant mouse *spastic*, a mutation in the β subunit leads to a reduction of glycine receptor expression within the spinal chord and other CNS areas and results in lethality 2 weeks after birth. In the case of the mutant mouse *spasmodic*, a point mutation in the α_1 subunit results in a reduced affinity of the receptors to glycine. *Oscillator* is allelic to *spasmodic*, but results in a complete loss of the α_1 subunit. Mice homozygous for *Oscillator* die after 3 weeks.

NMDA receptors comprise a family of ionotropic glutamate receptors that play a role in brain development, excitatory neurotransmission, synaptic plasticity and memory, and can bind both glutamate and glycine. The receptor is formed by assembly of NR1 subunits with any one of four NR2 subunits (NR2A-D), resulting in a $NR1_2$–$NR2_2$ complex. Glutamate binds to the NR2 subunits, and glycine to the NR1 subunits. In NR1, similar to the glycine receptor, Betz showed that a FXY motif is important for glycine binding.

In non-glutamatergic synapses, acetylcholine-gated cation channels often play a key role in synaptic transmission. The nerve-muscle endplate is probably the best-studied example of such a synapse. Steven Sine (Rochester, MN) focussed on insights into acetylcholine receptor (AChR) function as revealed by mutations that cause congenital myasthenic syndromes. The sites of the mutations ranged from the ACh binding site to the channel gate, and either increased or decreased the response to ACh. The mutation ε-P121L at one face of the ACh binding site slows the rate of channel opening and speeds the rate of channel closing to decrease the response to ACh. On the opposing face of the binding site, the mutation α-G153S causes marked flickering kinetics by slowing the rate of ACh dissociation to increase the response. Finally, the mutation ε-A411P, in an intracellular loop, produces a wide spectrum of channel gating kinetics likely due to distortion of the global energy landscape governing gating. Sine's mutational analysis illustrates the synergy between basic and clinical sciences to advance understanding of fundamental mechanisms as well as treatment of disease.

While roles of glutamate- and acetylcholine-gated cation channels in the CNS are very well established, this has been proven difficult for ATP-gated cation channels, although many studies have shown their expression in the CNS. Oleg Krishtal (Kiev, Russia) showed that, in CA1 hippocampal pyramidal neurons, around 20% of the excitatory activity depends on different subtypes of purinergic P2X receptors. Krishtal suggested that the function of P2X receptors could be to act as frequency filters, preventing long-term potentiation (LTP), a process required for the generation of 'memory'. While his data at present do not link an ion channel with a particular disease it is exciting news that P2X receptor functions in the CNS.

Hyperpolarization- and cyclic nucleotide-operated cation channels

Hyperpolarization-activated cation (HCN) currents (I_h) can be detected in a variety of cells including retinal photoreceptors, cardiac pacemaker cells, central and peripheral neurons and taste buds. Martin Biel (München, Germany) and David Stevens (Homburg, Germany) presented data on the functional implications of HCN currents. Biel showed that in the cardiac sinoatrial node, the most prominently expressed HCN channel type is the type 4 variant, HCN 4, whereas neurons of the dorsal root ganglions and photoreceptors preferentially express HCN1. Like HCN2 and HCN3, HCN1 and HCN4 support I_h currents. However, they differ markedly in their activation kinetics. To study the role of HCN channels in pacemaker activity, one future approach will be to generate conditional KO-mice.

Taste cells are present in bundles of around twenty in taste buds and are responsible for transduction of the five different qualities of taste: sweet, sour, salty, bitter and umami. Stevens presented evidence that HCN1 and -4 play major roles in a subset of taste cells responsible for transduction of sour taste. By performing whole-cell measurements in slices of the rat tongue, Stevens could identify a subset of taste cells expressing I_h, a mixed cation current activated upon hyperpolarization and modulated by protons. Immunostaining revealed that HCN1 and -4 colocalize in those cells. Interestingly, cells that expressed HCN1 and/or -4 never expressed gustducin, a protein present in bitter and sweet tasting cells. This indicates that HCN1 and -4 are only present in specialized taste cells that are not responsible for sweet or bitter transduction. Heterologous expression of HCN1 and -4 in HEK 293 cells revealed a current very similar to I_h in taste cells. Taken together, these results indicate that HCN1 and -4 are responsible for I_h and the initial step in the sour signal transduction cascade.

Achromatopsia (total colour-blindness) is a rare genetic disease caused by mutations in the cone photoreceptor cyclic nucleotide-gated (CNG) channel. Reinhard Seifert (Jülich, Germany) gave a short overview of the clinical characterization of the disease and reported on the heterologous expression of mutant CNG channel subunits that have been identified in a subset of the patients. He showed that some of the mutations that have been identified lead to non-functional channels. Other mutations (e.g. T369S) that have been found in patients displaying residual colour vision (incomplete achromatopsia) do not completely abolish channel expression but, instead, induce complex alterations in the electrophysiological properties of the respective channels.

Voltage-operated chloride channels

Thomas Jentsch (Hamburg, Germany) discussed the functional roles of the chloride channels CLC3, -5 and -7 in intracellular organelles of healthy subjects and in disease. CLC3 is expressed in endosomes and synaptic vesicles, where it might control acidification. CLC3-deficient mice showed a complete degeneration of the hippocampus but could survive and even learn motor skills without it. Mutations in CLC5, which is mainly expressed in the kidney, lead to Dent's disease, exhibiting the symptoms of low molecular weight proteinuria and hypercalciuria. CLC5 deficient mice showed a strong reduction in the amount of the endocytotic receptor megalin present in the

proximal tubule and strongly reduced receptor-mediated and fluid-phase endocytosis. The endocytotic defect led to defective handling of the calciotropic hormones PTH and vitamin D, which can explain the changes in calcium and phosphate metabolism in Dent's disease. Osteopetrosis, a severe disease resulting in very dense bones in which the bone marrow is also replaced by bone material can be caused by mutations in the *CLC7* gene. Osteopetrosis is due to defective acid secretion by osteoclasts, which need the CLC7 channel to secrete Cl^- in parallel to H^+. In summary, the data presented by Jentsch stresses how important chloride channels are for the development and physiological function of many organs.

Perspectives

The 83rd International Titisee Conference on 'Ion channels in health and disease', organized by the Boehringer Ingelheim Fonds highlighted that many human diseases are linked to ion channel dysfunctions. The list of channelopathies is growing each month and detailed knowledge of the involvement of ion channels in physiology and pathophysiology should be very useful for new drug design and efficient gene therapy.

This meeting was designed to focus on the importance of ion channels for cellular functions under physiological 'healthy' conditions and on the correlation of ion channel malfunction and disease. During the meeting it became obvious that a detailed analysis of ion channel properties is required to understand their relevance in health and disease. Unfortunately, in many cases there is no one-to-one link between an ion channel protein and a certain disease. From the data presented it can be concluded that defects in the complex interactions between ion channels and other proteins can cause major diseases such as diabetes, cancer and heart disease. Because of the central role of ion channels it will become increasingly important to study their complex interactions in detail, and to relate those findings to physiological and pathophysiological aspects of cell signalling.

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