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# Channels Gone Bad: Reflections from a Tapas Bar

Meeting Report

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Channels regulate ion flow across membranes and are an essential component of cell function. Indeed, nearly all cell membranes contain ion channels, proteins with diverse roles, and sometimes highly complex behaviors. Channels are activated and inactivated by many signals and their function regulated by countless processes. Yet, beware of the aberrant channel. Channels that open when they shouldn't, channels that do not open very well or at all, channels that stay open too long, misplaced channels, lack of channels, too many channels; all these scenarios can have disastrous consequences. Under the auspices of the Instituto Juan March, a group of fifty channelologists gathered in March 2002 to discuss these issues in a "Channelopathies" workshop organized by Thomas Jentsch (Universität Hamburg, Germany), Antonio Ferrer-Montiel (Universidad Miguel Hernandez, Spain), and Juan Lerma (Instituto Cajal, Spain). For this meeting, channelopathies were defined to include inherited human diseases and transgenic or knockout models in mice (Figure 1). Among the many topics presented, attendees witnessed a complete lack of hippocampus in mice without a chloride channel, migraine headaches as a result of mutations in calcium channels, as well as diabetes, cancer, arrhythmia, and epilepsy associated with various forms of potassium channel dysfunction. Our hosts, Andres Gonzalez and Lucia Franco, strongly encouraged interactions among participants by a traditional ritual, awarding a wine prize for the attendee that made the most significant contribution, to be chosen by secret ballot by all registrants and speakers at the end of the proceedings. Discussions of the topics covered during the day were usually continued well into the evening with lively conversations at the many outstanding Tapas bars and excellent restaurants that surround the Instituto. The purpose of this Meeting Report is to provide a synopsis of the topics that were discussed at this highly stimulating meeting.

## Neurotransmitter Receptors

Peter Seeburg (Max-Plank-Institute for Medical Research, Germany) began the meeting with a provocative video clip of a genetic mouse model in which RNA editing of the glutamate receptor subunit GLUR-B had been silenced for the transcripts from one allele. The inducible production of unedited, and as a result, high conductance $Ca^{2+}$-permeable AMPA channels in these mice led to fulminant and lethal seizures. The target for this genetic manipulation was an intronic site on the GLUR-B gene that is the target structure for the RNA editing enzyme adenosine deaminase. Seeburg suggested that single amino acid substitutions within this intron could lead to substantial editing deficits. As such, it may be possible that seizure disorders may arise from nonedited GLUR-B subunits in the brain. Another well-known model for the genesis of epileptic seizures is a decrease in GABAergic function. Juan Lerma presented evidence that kainate receptors present on GABA-containing terminals mediate a decreased release of the inhibitory neurotransmitter, and through this mechanism, may account for the epileptogenic actions of kainate. Activation of presynaptic kainate receptors on GABAergic neurons seems to lead to the initiation of a G protein signaling cascade independent of ion channel permeation, a somewhat unusual role for an ionotropic receptor. Nat Heintz (HHMI, The Rockefeller University, New York) suggested that GluR$\delta$2 is coupled to autophagy through a novel protein complex. Autophagy is the bulk degradation of proteins in response to starvation. When not fully activated, autophagy may be beneficial for cells by recycling proteins, but prolonged activation can lead to cell death. Whether GLUR$\delta$2-mediated autophagy requires ion flux is unclear since no ligand that can activate ion permeation through this receptor has been identified. Concluding the glutamate receptor session, James McNamara (Duke University, Durham) linked circulating GLUR-3 antibodies to Rasmussen's encephalitis in humans. This disease is characterized by severe epileptic seizures and an unexplained progressive degeneration of one cerebral hemisphere. Rabbits immunized with GLUR-3 develop seizures, and serum taken from these animals destroys cortical cells by antibody activation of the complement membrane attack complex.

Two presentations on channelopathies involving the nicotinic acetylcholine receptor (nAChR) and the glycine inhibitory receptor provided excellent examples of specific mutation-induced deficits in function. Andrew Engel (Mayo Clinic, Rochester) presented studies on nAChR mutations that either increase or decrease receptor activity. Congenital myasthenic syndromes resulting from mutations that prolong channel opening lead to enhanced synaptic activity, depolarizing block, and endplate myopathy. These "slow-channel" syndromes respond to long-lived open channel blockers, which normalize the open state. In "fast channel" syndromes, channel activation episodes are abnormally brief and channel opening probability is reduced. These syndromes respond well to drugs that increase the synaptic response. Heinrich Betz (Max-Plank-Institut für Hirnforschung, Germany) indicated that hereditary startle disease has been linked in many afflicted families to point mutations in the $\alpha$1 subunit of the inhibitory glycine

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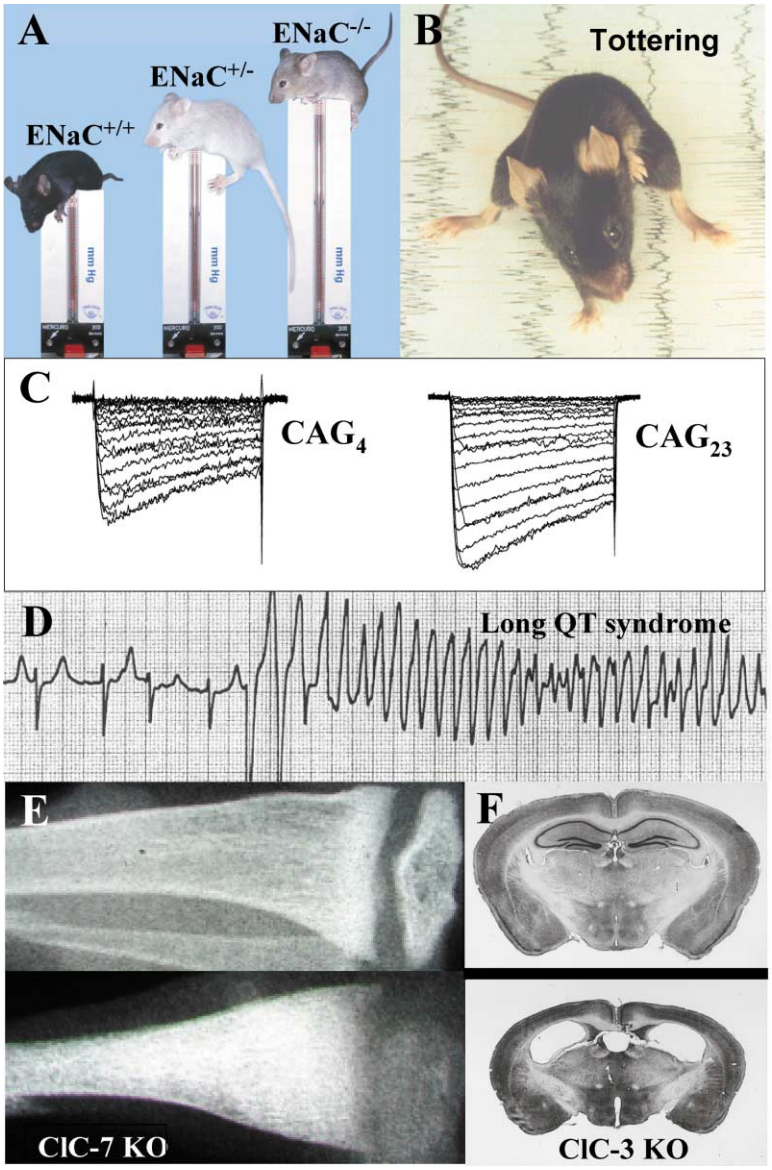


Figure 1. Potpourri of Channelopathies

(A) Mouse models are increasingly used to investigate the physiological consequences of mutations in, or knockout of, ion channel genes. For example, mutations in the epithelial $Na^+$ channel ENaC that cause Liddle's syndrome (renal hypertension) in humans can be modeled in transgenic mice (photo provided by B. Rossier). (B) Mutations in the $\alpha_{1A}$ $Ca^{2+}$ channel cause ataxia and epilepsy in mice (tottering phenotype) and mimic spinocerebellar ataxia in man (photo provided by J. Noebels). (C) Expanded trinucleotide (CAG) repeats in the $\alpha_{1A}$ $Ca^{2+}$ channel cause spinocerebellar ataxia type 6 (SCA6) in man and are associated with an increase in P/Q-type $Ca^{2+}$ channel current (Piedras-Renteria et al., 2001; copyright 2001, Society for Neuroscience). (D) Inherited mutations in HERG or KCNQ1 $K^+$ channels cause long QT syndrome and cardiac arrhythmia as depicted in this ECG tracing (provided by M. Vincent). (E and F) Knockout of $Cl^-$ channels in mice can lead to severe phenotypes. CLC-7 knockout causes osteopetrosis due to disruption of osteoclast function, mimicking the bone disorder in humans caused by mutations in this gene (Kornak et al., 2001). Knockout of CLC-3 in mice leads to a complete degeneration of the hippocampus (Stobrawa et al., 2001).

receptor. This rare disease is similar in many respects to well-known glycine receptor mutation disorders in the *spastic, spasmodic,* and *oscillator* mice. Glycine receptor mutations in these mice lead to decreased agonist affinity or decreased receptor expression. Betz went on to describe mice in which expression of the glycine receptor anchoring protein gephyrin has been silenced. Interestingly, these mice are also deficient in molybdenum cofactor (Moco) activity as gephyrin also subserves the function of a Moco-synthesizing enzyme.

Although not generally considered a classical neurotransmitter receptor, the vallinoid receptor ion channel VR-1 is responsible for the activation of sensory fibers by heat and noxious stimuli like capsaicin. Antonio Ferrer-Montiel presented his combinatorial chemistry efforts to generate VR-1 antagonists, one of which can prevent capsaicin-induced activation of sensory neurons, and is also effective in decreasing thermal nociception and hyperalgesia in vivo, without disrupting mechanical nociception.

## Calcium Channels

P/Q-type voltage-gated $Ca^{2+}$ channels are intimately involved with neurotransmitter release. Mutations of the $\alpha_{1A}$ subunit, from which these channels are comprised, can lead to several disorders including ataxia (Figure 1C), migraine, and epilepsy. Arn MJM van den Maagdenberg (Leiden University, The Netherlands) indicated that familial hemiplegic migraine (FHM), characterized by periods of unilateral paralysis, has been associated with mutations of the $\alpha_{1A}$ subunit. His analysis of single synapses of the neuromuscular junction of natural mouse mutants *tottering* and *rolling Nagoya* with different mutations in the same gene showed various func-

tional consequences, adding complexity to calcium channelopathies. Richard W. Tsien (Stanford University, Stanford, CA) suggested that a decrease in P/Q calcium channel function may be responsible for the symptoms associated with FHM and showed that transfection of various FHM-like mutated forms of $\alpha_{1A}$ in neurons obtained from mice deficient in the wild-type subunit ($\alpha_{1A}^{-/-}$) invariably led to smaller P/Q currents when compared to control. Daniela Pietrobon (University of Padova, Italy) described a similar result in transfected $\alpha_{1A}^{-/-}$ neurons. However, her detailed single channel analysis in transfected HEK293 cells revealed that FHM mutations invariably led to an increase in open channel probability, mostly apparent at lower voltages, resulting in higher calcium influx through single P/Q channels despite the reduced unitary conductance of some mutants. Tsien also suggested that too much $Ca^{2+}$ channel activity can lead to pathological abnormalities. For instance, in spinocerebellar ataxia type 6 (SCA 6), he and Piedras-Rentería find that a moderate CAG trinucleotide expansion in $\alpha_{1A}$ can lead to enhanced surface channel expression and $Ca^{2+}$ current amplitudes (Figure 1C), resulting in increased intracellular calcium and the ensuing pathogenesis characteristic of the disease.

Daniela Pietrobon presented evidence that missense $\alpha_{1A}$ mutations linked to episodic ataxia type-2 (EA-2) were always associated with a severe decrease in P/Q channel function. Dimitri Kullmann (University College London, United Kingdom) described two novel mutations, one sporadic and one inherited, of $\alpha_{1A}$ causing EA-2 accompanied by spike-wave epilepsy, reminiscent of several of the mouse mutants including the $\alpha_{1A}$ knockout. One of these mutations results in nonfunctional P/Q channels due to a truncation of the entire C terminus. Indeed, this channel acts as a dominant negative in expression studies, and Kullmann suggested that certain channelopathies might be due to functional dominant interfering effects of mutant channels.

Tsien presented evidence from Francisco Urbano and colleagues that N and R calcium channels could substitute for P/Q channels, albeit not identically, to sustain transmitter release at the neuromuscular junction in $\alpha_{1A}^{-/-}$ mice. Osvaldo Uchitel (Universidad de Buenos Aires, Argentina) indicated that although some transmitter release was present in these animals, the timing of release was variable and the observable synaptic failures were due to a decrease in the available pool of synaptic vesicles. Even though N and R channels are upregulated in $\alpha_{1A}^{-/-}$ mice, Pietrobon suggested that survival of cerebellar neurons depends specifically on a critical level of P/Q channel function, which cannot be fully compensated. The rescue of calcium channel function by compensatory mechanisms was discussed by Jeffrey Noebels (Baylor College of Medicine, Houston). Calcium entry through presynaptic $\alpha_{1B}$ channels can rescue impaired transmitter release at hippocampal synapses due to mutation of $\alpha_{1A}$ in the *tottering* mouse (Figure 1B). Novel interactions between the channel and non-pore-forming subunits can also rescue function. In the *lethargic* mutant, loss of the regulatory subunit $\beta_4$, the usual binding partner of $\alpha_{1A}$, is partially compensated due to the binding promiscuity of other $\beta$ subunits, such as $\beta_1$ and $\beta_3$. Noebels pointed out that absence of functional $\beta_4$ favors excitability changes in specific brain regions, such as deep cerebellar nuclei and thalamic nuclei, where $\beta_1$ and $\beta_3$ are not highly expressed. The neurological phenotype is thus critically defined by the pattern and degree of functional rescue.

**Potassium Channels**

Episodic ataxia type-1 (EA-1) has been associated with mutations in the delayed rectifier potassium channel Kv1.1. Kullmann presented results with various Kv1.1 mutations in which he correlated the degree of reduction in current amplitude with the severity of the disease. In a particularly severe form of EA-1, he described a Kv1.1 mutation leading to a truncation of most of the C terminus, which interferes with the assembly of the complete channel and results in intracellular accumulation of the defective protein. In addition to Kv1.1, mutations in other types of potassium channels can lead to a variety of human maladies. Michael Sanguinetti (University of Utah, Salt Lake City) linked potassium channel dysfunction to long QT syndrome (LQTS), a ventricular repolarization disorder that can lead to recurrent arrhythmias (Figure 1D) and sudden death. Mutations in either the KCNQ1 $K^+$ channel $\alpha$ subunit or its accessory $\beta$ subunit KCNE1 are responsible for about 50% of LQTS. Two other KCNQ proteins, KCNQ2 and KCNQ3, co-assemble to form neuronal M channels, and mutations in either cause benign familial neonatal convulsions. Alvaro Villarroel (Instituto Cajal, Spain) reported that calmodulin binds to two $\alpha$ helices in the C-terminal region of all KCNQ channels. Hence, modulation of KCNQ channel activity by intracellular $Ca^{2+}$ may be mediated through calmodulin interaction. Sanguinetti indicated that mutations in HERG $K^+$ channels also cause inherited LQTS and block of these channels is the major cause of drug-induced LQTS.

A provocative presentation by Walter Stühmer (Max-Planck-Institute for Experimental Medicine, Germany) highlighted the potential role of EAG channels in cancer. Stühmer reported that the voltage dependence and ionic selectivity of the EAG channel is dramatically influenced by the cell cycle. Interestingly, EAG-transfected cell lines grow faster than control, and lose contact inhibition, growth factor dependence, and substrate attachment requirement. Furthermore, CHO cells transfected with EAG are able to induce tumors when implanted in mice and inhibition of the channel in human tumor cell lines blocks proliferation. Stühmer concluded by suggesting that these channels would be ideal targets for novel antineoplastic agents. Elias Aizenman (University of Pittsburgh, Pittsburgh) suggested that delayed rectifier potassium channels might also be therapeutic targets to prevent neuronal cell death. Aizenman implicated a p38-dependent increased activation of potassium channels following the liberation of intracellular $Zn^{2+}$ in an oxidative stress model of neuronal apoptosis. Hella Lichtenberg (Universität Bonn, Germany) presented a method for screening compound libraries for $K^+$ channel blocking activity. The procedure involves integration of a mammalian $K^+$ channel gene into the genome of yeast devoid of endogenous $K^+$ transporters. As yeast lacking their endogenous transport system require the expressed channels to survive, the assay measures growth rates in the presence of drugs that potentially reduce $K^+$ channel function.

ATP-sensitive $K^+$ channels ($K_{ATP}$ channels) provide an important link between cell metabolism and electrical activity in a large variety of cells. In pancreatic β cells, $K_{ATP}$ channels mediate insulin secretion by closing in response to increased metabolism. Frances Ashcroft (University of Oxford, UK) indicated that the pancreatic β cell $K_{ATP}$ channel is a 4:4 heteromeric complex of the pore forming inwardly rectifying Kir6.2 subunit and the metabolic sensing subunit SUR1. Mutations in either Kir6.2 or SUR1 can lead to a loss of function and persistent insulin secretion.

**Chloride Channels, Epithelial Transport**

The CLC family of $Cl^-$ channels in humans is composed of nine genes, all encoding ten transmembrane domain pore-containing proteins that assemble as dimeric "double-barrel" channels. At the plasma membrane, $Cl^-$ channels stabilize membrane potential and regulate cell volume. In contrast, chloride channels in organelles provide the electrical shunt that is critical for the proper functioning of the $H^+$-ATPase that acidifies vesicles. Thomas Jentsch introduced three knockout models of intracellular $Cl^-$ channels, all with dramatic phenotypes. Mice deficient in ClC-5 channels residing in proximal tubule endosomes are deficient in fluid-phase and receptor-mediated endocytosis, as well as in endocytosis of hormones that regulate the expression of other proteins, such as Na/phosphate cotransporter. Mutations in ClC-5 have been associated with Dent's disease in humans, a disorder characterized by low molecular weight proteinuria, as well as kidney stones due to hypercalciuria and hyperphosphaturia. Mice deficient in ClC-7, critical for the acidification of lysosomes, develop osteopetrosis (Figure 1E) due to the inability of osteoclasts to degrade bone. Significantly, some humans with severe osteopetrosis have mutations in ClC-7. Astonishingly, disruption of another chloride channel, ClC-3, which is involved with acidification of synaptic vesicles, leads to a complete loss of the hippocampus (Figure 1F).

Mutations of the gene encoding the CFTR $Cl^-$ channel cause cystic fibrosis in humans. However, the mechanisms underlying regulation of CFTR channel activity are not well understood. CFTR has two nucleotide binding domains, two membrane-spanning domains, and an R domain, which functions as the major physiologic regulator of the channel. Michael Welsh (HHMI, University of Iowa, Iowa City) focused on how multiple phosphoserines in the R domain contribute to channel activity. Welsh showed data suggesting that the R domain has a random coil structure regardless of its phosphorylation state. This may begin to explain earlier apparently contradictory observations on how phosphorylation regulates the channel. The discussion shifted to the amiloride-sensitive $Na^+$ channel, responsible for sodium absorption in the kidney (ENaC). Bernard Rossier (Universite Bugnon, Switzerland) indicated that gain or loss of function of mutations in the subunits that encode the epithelial Na channel ENaC can lead, respectively, to hypertension or hypotension in afflicted individuals or transgenic mice (Figure 1A). Rossier also presented evidence that a series of membrane bound channel-activating proteases (CAPs) regulate ENaC activity by increasing open channel probability without enhancing surface expression. As CAPs and other modulators provide a wide dynamic range for altering ENaC function, Rossier suggested that novel antihypertensive drugs might arise by targeting these regulatory pathways.

**Gap Junction Channels**

Connexins represent the principal molecular component of gap junction channels. Klaus Willecke (Institüt fur Genetik, Germany) presented several mouse models in which specific connexins had been deleted. One of these was connexin26, which is defective in half of nonsyndromic profound prelingual hereditary deafness cases in Caucasian populations. He presented a conditional knockout of Cx26 in the inner ear, resulting in hearing impairment with apoptosis in the cochlear neuroepithelium. X-linked Charcot-Marie-Tooth disease is caused by mutations in Cx32, and Luis Barrio (Hospital Ramon y Cajal, Spain) showed that the effects of distinct mutants located in the carboxy-terminal domain of the molecule varied depending on the type and location of the mutation. Certain mutants were null whereas others formed hemichannels, but had reduced efficiency for assembly into full channels. Synchronization of inhibitory pathways might be mediated by electrical coupling between GABAergic interneurons. Hannah Monyer (University of Heidelberg, Germany) presented evidence that mice deficient in Cx36, which is highly enriched in GABAergic cells, leads to pronounced deficits in oscillatory γ activity as a result of a lack in connectivity among inhibitory neurons. Monyer also presented data regarding the successful generation of transgenic mice using bacterial artificial chromosomes. In the mice, specific subsets of GABAergic interneurons have been labeled, thus facilitating the study of specific interneurons at the cellular and system level in the slice preparation and in vivo.

**Concluding Remarks**

Channelopathies, as currently defined, are associated with inherited mutations in ion channels and sometimes with autoimmune disorders that affect channel function. However, otherwise normal channels, when improperly activated or localized, might also be at the root of many human maladies. Obvious examples presented here included $K^+$ channels in cancer and GLUR-δ2-mediated autophagy in neurodegeneration. Although this is not a new concept (e.g., glutamate receptor overactivation in excitotoxicity), can the definition of a channelopathy be expanded to include these phenomena? Classifying a field clearly does not dictate the direction individual investigators may take in their research, but it does promote interactions among scientists working on what may superficially appear as very disparate areas. As was most evident in the meeting in Madrid, this "cross-pollination" not only can serve to cement basic principles across a wide range of disciplines, but also provides an invaluable learning opportunity for scientists at all stages of their career.

By the way, Dick Tsien got the wine.

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