Instituto Juan March de Estudios e Investigaciones

136

CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Channelopathies

Organized by

T. J. Jentsch, A. Ferrer-Montiel and J. Lerma

F. M. Ashcroft
L. C. Barrio
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Introduction

T. J. Jentsch, J. Lerma and A. Ferrer-Montiel

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Ion channels play a critical role in the physiology of the nervous system. These molecules are fundamental in the generation of membrane potentials that are the essence of neuronal signalling. Although initially thought to be specific signalling molecules of the nervous system, it is known since long that ion channels are also present on non-neuronal cells such as lymphocytes or sperm cells where they play fundamental roles in cellular proliferation and differentiation. Thus, ion channels are widely distributed in neuronal and non-neuronal tissues. Furthermore, they are present in the plasma membrane, as well as in the membrane of intracellular organelles. The importance of these molecular devices in physiology and pathology is further highlighted by the discovery that they are the targets of a large number of toxins and drugs.

Ion channels are pores in the cell membranes that allow the flow of ions across the lipid bilayer, causing eventually a depolarization or hyperpolarization of the cell. These devices conduct ions down their electrochemical gradient at extremely rapid rates of up to 100,000,000 ions per second. This high permeation rate is often accompanied by an exquisite selectivity to one or more ions. In addition to these two properties, ion channels are controlled by gating mechanisms which involve a conformational change of the protein in response to specific stimuli. Gated channels can open or close rapidly in response to different signals, including voltage, chemical transmitters, heat, and pressure or stretch. It should be noted that non-gated channels also exist and significantly contribute to define the resting potential, as well as to cell-cell communication. Therefore, ion channel are a heterogeneous family of proteins that can be classified attending to their gating mechanism and/or ionic selectivity.

Structurally, ion channels are large integral membrane proteins that have a central pore that spans the entire width of the plasma membrane. They are most often oligomeric proteins formed by identical or different subunits organized around a central axis of symmetry. Channel subunits may be encoded by a single gene (homo-oligomers) or by different genes (hetero-oligomers). This molecular complexity is further expanded by the existence of accessory subunits essential for channel function. The progress of molecular biology along with electrophysiology has allowed the precise characterization of ion channel activity in terms of their underlying protein structure. Recently, the high-resolution X-ray crystallographic structure for a K^+ and a Cl^- channels has been reported, providing information on channel permeation and selectivity at an unprecedented detail.

A central question arises: What happens when ion channels do not work properly? Recent advances in medical genetics, biochemistry, molecular biology and electrophysiology have contributed to unravel the central role played by ion channel dysfunction in several human pathologies. Indeed, malfunction of these molecular machines may result in a variety of neurological and non-neurological disorders that span from myopathies to epilepsy and even bone disease. All these disorders of channel function are called channelopathies. These disorders may be caused by genetic alterations or by toxins or by autoimmunity. Inherited channelopathies may be due to gain-of-function mutations, or loss of function which in some cases requires a dominant negative behaviour of a mutated subunit. A characteristic of some channelopathies is the intermittent or episodic nature of the symptoms, presumably because significant mutations may have profound consequences in channel function leading to a lethal phenotype.

The increasingly known incidence of channelopathies in humans, along with the emergence of new information on the molecular and cellular causes underlying disease

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phenotypes, prompted the organization of this specific workshop under the auspices of the Fundación Juan March. The aim of the workshop was to provide an update of our current knowledge on genetic and autoimmune channelopathies. The meeting was organized in specific sessions that covered most aspects related to channel dysfunction and its relationship with human or animal disorders. The session 1 discussed the consequences of glutamate receptor channel dysfunction. The session 2 focused on calcium channels that have been critically involved in diverse human diseases. A discussion on malfunction on excitation (potassium channels and acetylcholine receptors) and inhibition (glycine-gated channels) ensued in session 3. An interesting session 4 was dedicated to describe involvement of non-neuronal channels in the aetiology of an increasing number of disorders including cystic fibrosis, hypertension, Dent's disease and cancer. And last, but not least, in session 5 the underlying mutations and functional alterations underlying connexinopathies were debated. In addition, the role of GABAergic interneurones in network synchrony and oscillatory activity in the brain, and the involvement of vanilloid receptor 1 (TRPV1) in inflammatory pain was presented. An outcome of this meeting was the realization that ion channels are not simple molecular devices that allow the flux of ions. An emerging concept was that these proteins are also important components of supramolecular complexes revealed as essential for cellular signalling.

Thomas J. Jentsch, Juan Lerma and Antonio Ferrer-Montiel

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Session 1: Glutamate receptors
Chair: Richard W. Tsien

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Myoclonic seizures in a conditional mouse model for epilepsy

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Early-onset epilepsy starting at postnatal day 14 (P14) can be generated in mice by overexpression in the central nervous system of high-conductance Ca^{2+}-permeable AMPA receptor channels, resulting from Q/R site-unedited GluR-B transcripts (Brusa et al., 1995; Feldmeyer et al., 1999). We now performed a study designed to correlate the generation of seizure activity with induced deficiency of GluR-B Q/R site editing in forebrain neurons at different postnatal times. We employed pharmacological (phenobarbital; doxycycline; RU486) and genetic (Cre recombinase; Cre-progesterone receptor fusion; ROSA 26 *lacZ* indicator mice) tools to regulate induction and effect of Q/R site-unedited GluR-B in the forebrain and to visualize neurons in which the induction had occurred. Mice between P50 - 70 were monitored by video and telemetry-based EEG recordings around the clock. The video recordings revealed jerks leading to tonic-clonic attacks, and the corresponding EEG recordings satisfied the criteria for myoclonic seizures. The onset and severity of this phenotype depended on the forebrain region in which expression of Q/R site-unedited GluR-B was induced. Our study identifies the CA3 region and dentate gyrus of hippocampus and cortical layers 5 and 6 as candidate regions that could serve as potential epileptic foci in this epilepsy model.

References:

Brusa, R. et al. *Science* 270, 1677 (1995).
Feldmeyer, D. et al. *Nat. Neurosci.* 2, 57 (1999).

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Role of kainate receptors in epilepsy generation

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Alterations of glutamatergic neurotransmission have been related to the neuronal damage observed after episodes of ischemia and hypoglycemia, as well as to the etiology of a series of neurological conditions including epilepsy, Alzheimer's disease, Huntington's chorea and amyotrophic lateral sclerosis. The cloning of a large number of glutamate receptor proteins and the discovery of their structural relationships have paved the way to most of our current understanding of the biophysical properties and the physiological role of each subtype in the mammalian brain. Of the glutamate receptor subtypes, NMDA, AMPA and kainate receptors, the latter is by far the less well understood. A decade ago, our understanding of the molecular properties of kainate receptors and their involvement in synaptic physiology was essentially null, despite of kainate administration in experimental animals induces seizures and patterns of neuronal damage closely resembling those observed in epileptics. For this and other reasons, it has become important to understand the physiology of these receptors in brain function. The discovery of a specific AMPA receptor antagonist, GYKI53655 (1), has made such studies feasible. A plethora of recent studies have shown that kainate receptors are key players in the modulation of transmitter release, important mediators of the postsynaptic actions of glutamate, and possible targets for the development of antiepileptic and analgesic drugs (2).

Although some effort has been done to link kainate receptor subunits (GluR5-7, KA1 and KA2) to human diseases, very few evidences are available to imply kainate receptor genotype variation in disorders such as Huntington's or Alzheimers' disease. However, it has been discovered that certain allelic variants of the human GluR5 subunit (GRIK1), but not of GluR6 (GRIK2), confer an increased susceptibility to the development of juvenile absence seizures (3). In addition, the level of kainate receptor mRNA seems to be altered in the hippocampus of patients suffering from temporal lobe epilepsy. The epileptogenic action of kainate has been well known for decades but the actual involvement of its specific receptors has just begun to be unravelled. The first indication that the activation of kainate receptors could elicit epileptiform discharges in vitro came from studies where low concentrations of the agonist, insufficient to activate AMPA receptors, could induce repetitive discharges in the hippocampus. In contrast, the results obtained after the systemic or the intracerebral administration of kainate in vivo have been more difficult to interpret and, although kainate administration has been widely used as a chemical model for human temporal lobe epilepsy, the evidence of the participation of kainate receptors has only accrued during the last years. Consistent with a role in epilepsy, we found that kainate depresses GABA inhibitory transmission in the rat hippocampus (4). GABA is the major inhibitory neurotransmitter in the brain, and its activity is crucial in maintaining neuronal excitability at normal levels.

In my talk, I will present data to support that the inhibition of GABA release is, at least in part, responsible for the epileptogenic action of kainate, since it activates specific kainate receptors on the GABAergic synaptic terminals.

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References:

1. Paternain, A.V., Morales, M. and Lerma, J. (1995) Selective antagonism of AMPA receptors unmasks kainate receptor-mediated responses in hippocampal neurons, **Neuron**, 14, 185-189

2. Lerma, J., Paternain, A.V., Rodríguez-Moreno, A., and López-García, J.C (2001) Molecular Physiology of Kainate Receptors. **Physiologial Reviews**. 81, 971-998

3. Sander T, Hildmann T, Kretz R, Fürst R, Sailer U, Bauer G, Schmitz B, Beck-Mannagetta G, Wienker T, and Janz D. Allelic association of juvenile absence epilepsy with a GluR5 kainate receptor gene (GRIK1) polymorphism. **Am J Med Genet** 74: 416-421, 1997.

4. Rodríguez-Moreno, A., Herreras, O. and Lerma, J. (1997) Kainate Receptors Presynaptically Downregulate GABAergic Inhibition in the Rat Hippocampus. **Neuron**, 19, 893-901, 1997

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Neurodegeneration in *Lurcher* mice

Heintz N.

Lurcher (*Lc*) is a spontaneous, semidominant mouse neurological mutation. Heterozygous lurcher mice (*Lc*/+) display ataxia due to a selective, cell-autonomous death of 90% of cerebellar Purkinje cells during postnatal development. Homozygous lurcher mice (*Lc*/*Lc*) die shortly after birth due to massive loss of mid- and hind-brain neurons during late embryogenesis. By positional cloning, we identified the mutations responsible for neurodegeneration in two independent *Lc* alleles as identical G-to-A transitions that change a highly conserved alanine to a threonine residue in transmembrane domain III of the mouse d2 glutamate receptor gene. *Lc*/+ Purkinje cells displayed a very high membrane conductance and a depolarized resting potential, indicating the presence of a large, constitutive inward current. Expression of the mutant $GluRd2^{Lc}$ protein in *Xenopus* oocytes confirmed these results, demonstrating that lurcher is an inherited neurodegenerative disorder resulting from a gain of function mutation in a glutamate receptor gene. Thus, the activation of neuronal death pathways in *Lc* mice may provide a physiologically relevant model for excitotoxic cell death.

Recent studies in the laboratory have identified a novel protein complex tethered to the C-terminus of the d2 glutamate receptor gene. Characterization of the activities of this complex demonstrate direct coupling of GluRd2 to proteins regulating macroautophagy, a cellular pathway often considered a second form of programmed cell death. These studies suggest that constitutive activation of $GluRd2^{Lc}$ receptors at the cell surface leads to activation of macroautophagy, which may contribute to death of Purkinje cells in *Lc*/+ mice. These findings, their relevance to other neurodegenerative diseases, and the role of d2 glutamate receptors in regulating synaptic plasticity in normal cells will be discussed.

References:

1. Zuo et al, Nature 1997 Aug 21;388(6644):716-7
2. Doughty et al, J Neurosci 2000 May 15;20(10):3687-94
3. Bursch, Cell Death Differ 2001 Jun;8(6):569-81
4. Klionsky and Emr, Science 2000 Dec 1;290(5497):1717-21

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Rasmussen's encephalitis and autoimmune attack on brain synapses

James O. McNamara

Rasmussen's encephalitis is an inflammatory disease of unknown cause that most commonly afflicts children in the first decade of life. It is characterized by severe epileptic seizures and the bizarre and unexplained progressive destruction of a single cerebral hemisphere. Standard therapy consists of surgical removal of the diseased hemisphere. Work in our lab has demonstrated the presence of humoral immune attack on neuronal antigens in this disease, in particular synaptic proteins including the GluR3 subtype of glutamate receptor. Analysis of a rabbit model *in vivo* together with mixed neuronal-glial cultures of embryonic rat cortex have demonstrated that anti-GluR3 destroys cortical cells in a complement dependent manner. The mechanism by which complement destroys these cells involves formation of the membrane attack complex, a heteromeric ion channel that inserts into the cell membrane and destroys the cell through osmotic lysis and ion flux down electrochemical gradients. These studies may provide insight into deleterious consequences of complement activation on CNS neurons in Rasmussen's encephalitis and diverse nervous system diseases including Alzheimer's.

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Development of a yeast-based screening system to enable the precise pharmacological/toxicological analysis of mammalian potassium channels expressed in yeast

Hella Lichtenberg

Yeast provide useful systems for drug testing in several ways. First, their use for growth-based assays for various chemical screens, second as model organisms for investigation of complex eukaryotic cellular processes, and third their use as recombinant systems for analysis and validation of therapeutic target proteins from higher eukaryotes.

A combined approach was utilised encompassing yeast genetics, molecular biology, eukaryotic K^+ channel research, physiological and biochemical protein expression analysis and electrophysiological analysis of yeast ion transport. *Saccharomyces cerevisiae* was chosen as major model organism because of its fully sequenced genome, susceptibility to genetic manipulation and availability of protocols for electrophysiological analysis.

Isogenic *S. cerevisiae* mutants carrying single and all possible combinations of potassium transporter mutations were constructed. Efforts have been directed at obtaining detailed phenotypic profiles of all yeast mutant strains. The strains have been subjected to a variety of analytical tests ranging form growth based and physiological assays to precise electrophysiological measurements. The identification of a yeast tok1 mutant phenotype, crucial to the expression of outward-rectifying channels, was finally successful.

The heterologous expression of single mammalian potassium channels involved two different strategies. The approach of plasmid borne expression used both, constitutive and inducible promoters. The other approach, applicable preferentially for *S. cerevisiae* involved the stable integration of the mammalian K^+ channel genes into the genome. In all cases, yeast mutant strains, devoid of endogenous K^+ transport activity, served as hosts for expression. In total, a variety of 10 different K^+ channel genes from divergent physiological classes with a focus on the human h-erg channel (involved in ventricular tachycardy), the rat homologue of the Kir2.1 inward-rectifying channel (involved in metabolic diseases) and the outward-rectifying r-eag channel (putatively involved in malignant transformation) have been expressed in appropriate yeast mutant strains and physiologically and biochemically analysed.

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Session 2: Calcium channels
Chair: Heinrich Betz

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Calcium channelopathies: too little or too much of a good thing?

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This talk will focus on channelopathies arising from defects in P/Q-type Ca^{2+} channels. These voltage-gated channels are abundant in the mammalian brain, particularly in cerebellar Purkinje and granule neurons, and are supported by the α_{1A} subunit ($Ca_V2.1$, encoded by CACNA1A, on human chromosome 19p13). They represent the principal Ca^{2+} entry pathway involved in triggering fast neurotransmitter release at excitatory and inhibitory synapses throughout the CNS. Alterations in α_{1A} can give rise to ataxia, migraine or epileptiform activity.

When the coding region of α_{1A} undergoes CAG trinucleotide repeat expansions, the result is spinocerebellar ataxia type 6 (SCA6), an autosomal dominant, progressive disease. Studying SCA6 is of general interest for understanding neurodegeneration produced by trinucleotide repeats.Unlike many other polyQ-carrying proteins, α_{1A} exhibits unusually small expansions, 21-30 repeats in SCA6 compared to 4-18 repeats in normal individuals. In approaching SCA6, we used both immunocytochemistry and electrophysiology to assay ion channel distribution and function following introduction of various human α1A cDNAs in HEK293 cells that stably co-expressed β_1 and $\alpha_2\delta$ subunits. Immunocytochemical analysis showed a rise in intracellular and surface expression of α_{1A} protein when CAG-repeat lengths reached or exceeded the pathogenic range. This gain at the protein level was not a consequence of changes in RNA stability. The electrophysiological behavior of α_{1A} subunits containing expanded (EXP) numbers of CAG repeats was compared against that of WT subunits. The EXP α1A subunits yielded functional ion channels that supported inward Ca^{2+} channel currents, with a sharp increase in P/Q Ca^{2+} channel current density relative to WT, a small shift in the voltage-dependence of activation, favoring greater current, but otherwise near-normal biophysical properties. Our data suggest that increased membrane protein in SCA6 may give rise to elevated channel activity and altered calcium homeostasis in a genetically dominant manner. The cytosolic accumulation of mutant α_{1A} protein we observed may also contribute to SCA6 pathogenesis.

In contrast to SCA6, which seems to arise from too much Ca^{2+} channel activity, the defect in familial hemiplegic migraine (FHM) is probably not enough. This seems to be the emerging pattern from work of D. Pietrobon and colleagues (this meeting) and from Yuqing Cao in our laboratory, who has transfected hippocampal neurons from mouse brain lacking α_{1A} with FHM mutant P/Q-type channels. The mutant α_{1A} subunits gave rise consistently to smaller P/Q currents than those produced by WT α_{1A} subunits.

To understand the whole animal consequences of deficiencies in P/Q-type Ca^{2+} entry in the most extreme case, we studied mice whose α_{1A}subunits were completely deleted (Jun et

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al., 1999; Piedras-Renteria et al., 2002). The absence of P/Q type Ca^{2+} channels causes both expected and unexpected changes. As anticipated, P-type and P/Q-type channels were eliminated from whole cell recordings of Ca^{2+} channel current in cerebellar neurons. Neurotransmission was rescued by participation of other Ca^{2+} channels of the Ca_V2 subfamily, notably N-type and R-type channels. Interestingly, at the mouse neuromuscular junction (NMJ), N-type-only transmission was significantly less steeply $[Ca^{2+}]_o$-dependent than R-type-only or wild type P/Q-only transmission. These differences were traced to differences in spatial proximity. R-type channels lie close to Ca^{2+} sensors for exocytosis and $I_{K(Ca)}$ channel activation, like the P/Q-type channels they replaced. In contrast, N-type channels were less well localized, but abundant enough to influence secretion strongly, particularly when action potentials were prolonged. Our data suggested that active zone structures may select among multiple Ca^{2+} channels in the hierarchy P/Q>R>N. The nonequivalence of various types of channels suggests that there may be "slots" for Ca^{2+} channels, formed by interacting protein components, with selectivity for the various α_1 subunits. The concept of slots carries with it the implication that the occupancy of a slot by a mutant α_{1A} subunit might prevent a normal α_{1A} from doing its job – a classical dominant negative mechanism.

The P/Q-type knockout animals also displayed additional effects at both peripheral and central synapses that might not have been anticipated. Developmental defects were seen in the timing of the migration of cerebellar granule neurons. At the α_{1A}-/- NMJ, changes in presynaptic function were associated with a significant reduction in the size of postsynaptic acetylcholine receptor clusters. Presynaptic transmitter release in the knockouts displayed several other differences from WT: a greater ability to withstand reductions in the Ca^{2+}/Mg^{2+} ratio, and little or no paired-pulse facilitation, both findings possibly reflecting compensatory mechanisms at individual release sites. A likely related form of synaptic compensation was observed at hippocampal synapses: a significant increase in miniature EPSC frequency. Using cDNA microarrays, we have found significant changes in the levels of transcripts in signaling pathways that support or modulate Ca^{2+} dependent transmitter release. Associated physiological experiments provided direct confirmation of the idea that a regulatory set-point in excitation-secretion coupling had been altered. These findings will be discussed in the context of molecular approaches to Ca^{2+} channelopathies.

References:

Jun, K., Piedras-Rentería, E.S., Smith, S.M., Wheeler, D.B., Lee, S.B., Lee, T.G., Chin, H., Adams, M.E., Scheller, R.H., Tsien, R.W. & Shin, H.-S. (1999). Ablation of P/Q-type Ca^{2+} channel currents, altered synaptic transmission and progressive, lethal ataxia in mice lacking the α_{1A} subunit. *Proc. Natl. Acad. Sci.*, 96-26, 15245-15250.

Klockgether T, Evert B (1998) Genes involved in hereditary ataxias. *Trends Neurosci* 21: 413-418.

Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SMG, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen G-JB, Hofker MH, Ferrari MD , Frants RR (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* 87: 543-52.

Piedras-Rentería, E.S., Watase, K., Zhuchenko, O., Zoghbi, H.Y., Lee, C.-C. & Tsien, R.W. (2001). Increased expression of α_{1A} Ca^{2+} channel currents arising from expanded trinucleotide repeats in spinocerebellar ataxia type 6 (SCA6). *J. Neurosci.* 21, 9185-9193.

Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A}-voltage-dependent calcium channel. *Nature Genet* 15: 62-69.

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Solving calcium channelopathy phenotypes in the CNS

Jeffrey L. Noebels

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Molecular genetic strategies have taken precise aim at the enigma of epilepsy, and the mechanisms underlying defects in some inherited hyperexcitability disorders of the brain are being solved. Predictably, mutations of voltage-gated Na+ and K+ ion channels that prolong membrane depolarization are a direct source of abnormal burst firing and neuronal synchronization in cortical circuitry. In contrast, spontaneous mutations of each of the four ($\alpha1$, β, γ, and $\alpha2\delta$) subunits of voltage-gated calcium channels reduce inward depolarizing currents, yet lead, paradoxically, to thalamocortical oscillations and absence seizures in mutant mice after three weeks of age. Our laboratory is interested in understanding how mutation of each subunit in the heterotetramer calcium channel complex leads to hyperexcitability, and why the signaling defects are confined to specific networks and appear at specific developmental stages.

Continuing analysis suggests that these calcium ion channelopathies are biologically distinct, and further classification provides a useful framework to understand the functional lesion they create in brain. *Simple* ($\alpha1$ subunit) channelopathies uniformly alter a single current type in neurons where the subunit is expressed, while *complex* (regulatory subunit) channelopathies alter multiple calcium current types. The *intragenic site* of rearrangement allows a second level of diversity, since loss of specific binding domains can alter cell-specific modulatory interactions, or allow novel subunit stoichiometries. The *cellular expression pattern* is critical, since while all of these channel subunit genes are widely localized in brain, the patterns of expression are non-overlapping, further increasing the diversity of excitability change.

Recently we have shown that selective excitability changes in the brain with a calcium channelopathy arise from a combination of all three of these factors. The phenotype of both simple and complex channelopathies arise from functional rescue of calcium currents, either by functional compensation (simple), or by novel post-transcriptional pairings with alternative subunits in the same family (complex). These rescuing subunits are expressed in some but not all cell types, and define the vulnerability of specific circuits. Thus selective expression and subunit reshuffling of related subunit family members provide two mechanisms for neuronal specificity and the emergence of a specific neurological phenotype in inherited brain ion channel disorders.

References:

Burgess, DL, Jones, J, Meisler, M, Noebels, JL Mutation of the Ca2+ channel β subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. ***Cell***, 1997, 88:385-392.

Burgess, DL, Biddlecome, GH, McDonough, SI, Diaz, ME, Zilinski, CA, Bean, BP, Campbell, KP, Noebels, JL Subunit reshuffling modifies N- and P/Q-type Ca^{2+} channel subunit compositions in lethargic mouse brain. ***Molecular and Cellular Neuroscience***, 1999, 13:293-311.

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Burgess DL and Noebels, JL Voltage-Dependent Calcium Channel Mutations in Neurological Disease. In: "Molecular and Functional Diversity of Ion Channels and Receptors". ***Annals of The New York Academy of Sciences***, New York, 1999, 868:199-212.

Qian, J and Noebels, JL Presynaptic Ca^{2+} influx at a mouse central synapse with Ca^{2+} channel subunit mutations. ***J. Neuroscience***, 2000, 20:163-170.

Burgess, DL, Gefrides, L, Foreman, PJ, Noebels, JL A Cluster of Three Novel Ca^{2+} Channel γ Subunit Genes on Chromosome 19q13.4: Evolution and Expression Profile of the γ Subunit Gene Family. ***Genomics***, 2001 71:339-350.

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Ca channel, human migraine, ataxia

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Migraine is a common, chronic, disabling, neurovascular disorder, typically characterised by recurrent attacks of severe headache and autonomic symptoms (migraine without aura) in one third of patients attacks may also be associated with neurological aura symptoms (migraine with aura). Although the mechanisms underlying the headache phase are reasonably well understood, hardly anything is known about how migraine attacks begin.

Genetic and non-genetic factors clearly are involved in migraine and the identification of "migraine genes" may help in understanding migraine pathophysiology.

Several years ago, we discovered the first migraine gene (CACNA1A) on chromosome 19p13, encoding a neuronal calcium channel subunit in families with familial hemiplegic migraine (FHM), which is a rare autosomal dominantly inherited subtype of MA (1). Mutations have been identified in the CACNA1A gene that are associated with a spectrum of neurological phenotypes, ranging from relatively mild episodic disorders, such as migraine, FHM, episodic ataxia type 2, to more severe permanent symptoms, such as progressive cerebellar ataxia and severe atrophy, sometimes associated with coma (2). Genetic evidence exists that the same locus is involved in the frequent forms of migraine as well (3). At this moment, it is of importance to investigate the functional consequences of the mutations in order to understand how they cause the disease. Existing natural mouse models and the generation of transgenic knock-in models will provide novel avenues to unravel migraine pathophysiology. Recently, we have already shown that the consequences of Cacna1a mutations on channel function in neuromuscular junction can be studied in *tottering* mice (4).

As approximately 50% of the reported FHM families have been assigned to chromosome 19p13 it is of importance to identify additional genes. Initiatives are undertaken to perform these kind of analyses.

References:

1. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen GJ, Hofker MH, Ferrari MD, Frants RR. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* 87:543-552 (1996).
2. Kors EE, Terwindt GM, Vermeulen FL, Fitzsimons RB, Jardine PE, Heywood P, Love S, Van den Maagdenberg AMJM, Haan J, Frants RR, Ferrari MD. Delayed cerebral edema and fatal coma after minor head trauma: Role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. *Ann Neurology* 49:753-760 (2001).
3. Terwindt GM, Ophoff RA, van Eijk R, Vergouwe MN, Haan J, Frants RR, Sandkuijl LA, Ferrari MD. Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. *Neurology* 56:1028-1032 (2001).
4. Plomp JJ, Vergouwe MN, Van den Maagdenberg AMJM, Ferrari MD, Frants RR, Molenaar PC. Abnormal transmitter release at neuromuscular junctions of mice carrying the tottering alpha(1A) Ca(2+) channel mutation. *Brain* 123:463-471 (2000).

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Functional consequences of mutations in CACNA1A linked to familial hemiplegic migraine and episodic ataxia type 2

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P/Q ($Ca_V2.1$) calcium channels are expressed throughout the brain in most presynaptic terminals, where they play a prominent role in controlling neurotransmitter release, and also in the soma and dendrites of most central neurons; their expression is particularly high in the cerebellum. Mutations in CACNA1A, the gene encoding the pore-forming α_{1A} subunit of P/Q channels, cause a group of dominantly inherited human neurologic disorders, including familial hemiplegic migraine (FHM), episodic ataxia type-2 (EA-2) and spinocerebellar ataxia type 6. Some families, in addition to the episodic attacks of migraine or ataxia, show progressive cerebellar atrophy and ataxia (PCA). Recently, a missense mutation in the same gene has been associated with a syndrome in which trivial head trauma can trigger severe cerebral edema and coma, after a lucid interval, with FHM attacks only in some subjects.

Recessive mutations at the mouse orthologue cause ataxia, episodic dystonia, cerebellar atrophy and absence epilepsy. By combining whole-cell and single channel current recordings on HEK293 cells transfected with wild-type and mutant human $Ca_V2.1$ channels, we studied the functional consequences of two missense mutations linked to EA-2 (with PCA), and of five missense mutations linked to FHM (with and without PCA), one located in the voltage sensor and four located in different pore regions (Hans et al., 1999; Guida et al., 2001). We also studied the functional consequences of the mutation S218L, associated with delayed coma-FHM. The FHM mutations, including S218L, affect in a complex manner both the biophysical properties and the density of functional channels in the membrane, leading to the prediction of either a decreased (loss-of-function) or increased (gain-of-function) Ca2+ influx into neurons, depending on the mutation. However, considering the single channel Ca2+ influx (as measured by the product of single channel current and open probability) all FHM mutations lead to a predicted higher Ca2+ influx at low voltages, near and below the voltage threshold of activation of $Ca_V2.1$ channels (gain of function). This effect appears particularly large for the S218L mutation. On the other hand, the EA-2 mutations lead to either complete or severe reduction of Ca2+ influx through human $Ca_V2.1$ channels. Our data support the conclusion that severe loss of $Ca_V2.1$ channel function underlies the pathophysiology of EA2, and suggest the working hypothesis that the permanent cerebellar symptoms of progressive ataxia and cerebellar atrophy, common to a subset of mutations linked to FHM and EA2, are specifically linked to loss-of-function of cerebellar $Ca_V2.1$ channels. The neurological phenotype of cacna1a knockout mice, completely lacking P/Q channels, is consistent with this hypothesis. Indeed, the null mice are severely ataxic/dystonic and show selective progressive cerebellar degeneration in a specific pattern, characterized by loss of Purkinje cells in parasagittal stripes and graded loss of granule cells more severe in the anterior lobe (Fletcher et al., 2001). Heterozygous null mice have no phenotype in terms of motor performance, cerebellar neuroanatomy or EEG, but show a 50% reduction of P/Q-type current density in cerebellar granule cells, without compensation by other calcium channel types. In contrast, in cerebellar granule cells of homozygous null mice the elimination of the P/Q current is partially compensated by an increased density of L- and N-type Ca channels.

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Since L-type channels activate at more negative voltages than P/Q-type, the compensation is complete at low voltages. Measurements of intracellular Ca2+ transients following depolarizing stimuli in cerebellar slices of null mice using confocal microscopy, confirmed the electrophysiological data obtained for cerebellar granule cells in culture, and revealed a similar upregulation of L-type channels in Purkinje cells. No significant difference in total Ca2+ transients and nimodipine-sensitive Ca2+ transients were found in cerebellar granule cells of the anterior and posterior vermis. Thus, we can exclude that the different susceptibility to death of neurons in the anterior and posterior vermis is due to a different ability to compensate the loss of P/Q channels. Our data are consistent with the conclusion that survival of a subpopulation of cerebellar neurons requires a critical level of P/Q channel function that cannot be compensated by other calcium channel types.

References:

1. C. Fletcher, A. Tottene, V.A. Lennon, S.M. Wilson, S.J. Dubel, R. Paylor, D.A. Hosford, L. Tessarollo, M.W. McEnery, D. Pietrobon, N.G. Copeland and N. A. Jenkins
"Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity."
FASEB J. (2001) 15, 1288-1290
2. S. Guida, F. Trettel, S. Pagnutti, E. Mantuano, A. Tottene, L. Veneziano, T. Fellin, M. Spadaio, K. Stauderman, M.E. Williams, S. Volsen, R.A. Ophoff, R.R. Frants, C. Jodice, M. Frontali and D. Pietrobon
"Complete loss of P/Q calcium channel activity caused by CACNA1A missense mutation carried by episodic ataxia type 2 patients."
Am. J. Hum. Genet. (2001) 68, 759-764
3. M. Hans, S. Luvisetto, M.E. Williams, M. Spagnolo, A. Urrutia, A. Tottene, P.F. Brust, E.C. Johnson, M.M. Harpold, K.A. Stauderman and D. Pietrobon
"Functional consequences of mutations in the human α1A calcium channel subunit linked to familial hemiplegic migraine."
J. Neurosci. (1999) 19, 1610-1619.

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Mechanisms underlying phenotypic variability of the episodic ataxias

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Two human forms of episodic ataxia (EA) are known to result from dominantly inherited mutations of ion channels [1]. EA-1 is characterised by brief episodes of cerebellar incoordination, and interictal neuromyotonia caused by ectopic action potentials in motor axons. It is linked to missense mutations of KCNA1, which codes for the $K_V1.1$ potassium channel α subunit [2]. EA-2, on the other hand, is characterised by relatively more prolonged episodes of ataxia, and although neuromyotonia does not occur, many patients have a mild but progressive cerebellar degeneration causing nystagmus and gait impairment. It is caused by missense or splice site mutations of CACNA1A, which codes for the α_{1A} subunit of $Ca_V2.1$, the P/Q-type calcium channel [3]. Since the original identification of these ion channels as the cause of EA1 and EA2, a broader range of phenotypes has emerged in association with distinct mutations. KCNA1 mutations have been identified in families with neuromyotonia without ataxia, or with seizures [4,5]. Kindreds also differ with respect to the severity and drug-responsiveness of the disorder. As for CACNA1A, distinct mutations are associated with a broad spectrum of disorders, ranging from familial hemiplegic migraine to a relatively pure progressive cerebellar degeneration [1].

$K_V1.1$ is relatively ubiquitous in the CNS and in myelinated motor axons, where it is thought to contribute to repolarisation following action potentials. Distinct disease-associated mutations are associated with variable alterations of activation threshold and kinetics, and generally lead to a decrease in maximal current amplitude [4-14]. However, the degree of reduction in current amplitude ranges from imperceptible to complete with dominant negative effects on the wild type allele. We have recently shown that the degree of reduction in current amplitude is correlated with the severity of the phenotype [4,14]. One particularly severe mutation, associated with drug-resistant EA1, is caused by a premature stop codon that is predicted to truncate most of the C-terminus (R417stop). By combining a number of different methods (current amplitude measurements, single channel recordings, pharmacological tagging and titration of tetraethylammonium sensitivity, and confocal imaging of GFP-tagged subunits), we have argued that the R417stop mutation interferes with channel tetramerisation and trafficking, as well as altering the voltage dependence and activation rate of heteromeric channels [14] (see also [15]). Depending on the severity of these phenomena, other mutations may interfere with the normal role of $K_V1.1$ in membrane repolarisation in the cerebellum, motor axons and forebrain. A fuller understanding of the disease mechanism may require investigation of interactions with other K_V1 and β subunits.

An important insight into the role of α_{1A} comes from examining spontaneous or experimental murine mutations of this subunit. Several mouse mutants share in common with the knockout model a combination of ataxia and spike and wave epilepsy reminiscent of absence epilepsy [16-18]. However, apart from some evidence of linkage disequilibrium of polymorphisms with idiopathic generalised epilepsy [19], there has been no direct evidence that human epilepsy results from CACNA1A mutations. We have recently reported the first sporadic case of EA2 in combination with absence epilepsy and learning difficulties with a

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previously unreported heterozygous CACNA1A mutation [20]. This mutation (R1820stop) also results in truncation of the entire C-terminus, and is downstream of premature stop codons and splice site mutations associated with uncomplicated EA2. We have also identified a family in whom another missense mutation co-segregates with a similar combination of EA2 and absence epilepsy (unpublished). The R1820stop mutation leads to complete loss of function upon expression in *Xenopus* oocytes [20]. Surprisingly, when co-expressed with wild type α_{1A}, we observed a dominant negative effect on current amplitude, with no effect on kinetics. The mechanism underlying this dominant negative effect is unclear, but we have argued against sequestration of accessory subunits, on the basis that it persists in the face of over-expression of $\alpha_2\delta$ and β_4, or substitution of β_3 for β_4, or expression of the α_{1A} subunits without accessory subunits. A similar dominant negative effect has recently been reported for experimental truncations of the related calcium channel subunit α_{1B}[21].

The phenotypic spectrum of KCNA1 and CACNA1A mutations will no doubt continue to grow. Elucidation of the mechanisms may be helped by the introduction of human disease-associated mutations into mice.

References:

1. Kullmann, D. M., Rea, R., Spauschus, A. & Jouvenceau, A. The inherited episodic ataxias: how well do we understand the disease mechanisms? *Neuroscientist* **7**, 80-8. (2001).

2. Browne, D. L. *et al.* Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1 [see comments]. *Nat Genet* **8**, 136-40 (1994).

3. Ophoff, R. A. *et al.* Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* **87**, 543-52 (1996).

4. Eunson, L. H. *et al.* Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability [In Process Citation]. *Ann Neurol* **48**, 647-56 (2000).

5. Zuberi, S. M. *et al.* A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain* **122**, 817-25 (1999).

6. Adelman, J. P., Bond, C. T., Pessia, M. & Maylie, J. Episodic ataxia results from voltage-dependent potassium channels with altered functions. *Neuron* **15**, 1449-54 (1995).

7. Zerr, P., Adelman, J. P. & Maylie, J. Episodic ataxia mutations in Kv1.1 alter potassium channel function by dominant negative effects or haploinsufficiency. *J Neurosci* **18**, 2842-8 (1998).

8. Zerr, P., Adelman, J. P. & Maylie, J. Characterization of three episodic ataxia mutations in the human Kv1.1 potassium channel. *FEBS Lett* **431**, 461-4 (1998).

9. D'Adamo, M. C., Liu, Z., Adelman, J. P., Maylie, J. & Pessia, M. Episodic ataxia type-1 mutations in the hKv1.1 cytoplasmic pore region alter the gating properties of the channel. *Embo J* **17**, 1200-7 (1998).

10. D'Adamo, M. C., Imbrici, P., Sponcichetti, F. & Pessia, M. Mutations in the KCNA1 gene associated with episodic ataxia type-1 syndrome impair heteromeric voltage-gated K(+) channel function. *Faseb J* **13**, 1335-45 (1999).

11. Boland, L. M., Price, D. L. & Jackson, K. A. Episodic ataxia/myokymia mutations functionally expressed in the Shaker potassium channel. *Neuroscience* **91**, 1557-64 (1999).

12. Bretschneider, F., Wrisch, A., Lehmann-Horn, F. & Grissmer, S. Expression in mammalian cells and electrophysiological characterization of two mutant Kv1.1 channels causing episodic ataxia type 1 (EA-1). *Eur J Neurosci* **11**, 2403-12 (1999).

13. Spauschus, A., Eunson, L., Hanna, M. G. & Kullmann, D. M. Functional characterization of a novel mutation in KCNA1 in episodic ataxia type 1 associated with epilepsy. *Ann N Y Acad Sci* **868**, 442-6 (1999).

14. Rea, R., Spauschus, A., Eunson. L., Hanna, M. G. & Kullmann, D. M. Variable K+ channel subunit dysfunction in inherited mutations of KCNA1. *J Physiol* **538**, 5-23 (2002).

15. Manganas, L. N. *et al.* Episodic ataxia type-1 mutations in the Kv1.1 potassium channel display distinct folding and intracellular trafficking properties. *J Biol Chem* **25**, 25 (2001).

16. Fletcher, C. F. *et al.* Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* **87**, 607-17 (1996).

Instituto Juan March (Madrid)

17. Jun, K. *et al.* Ablation of P/Q-type Ca(2+) channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)- subunit. *Proc Natl Acad Sci U S A* **96**, 15245-50 (1999).
18. Fletcher, C. F. *et al.* Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity. *Faseb J* **15**, 1288-90 (2001).
19. Chioza, B. *et al.* Association between the alpha(1a) calcium channel gene CACNA1A and idiopathic generalized epilepsy. *Neurology* **56**, 1245-6. (2001).
20. Jouvenceau, A. *et al.* Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet* **358**, 801-807 (2001).
21. Raghib, A. *et al.* Dominant-negative synthesis suppression of voltage-gated calcium channel cav2.2 induced by truncated constructs. *J Neurosci* **21**, 8495-504. (2001).

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Session 3: Excitation & Inhibition
Chair: Frances M. Ashcroft

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Molecular mechanisms of inherited and acquired long QT syndrome

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Inherited long QT syndrome

Long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmias and sudden death. The most common form of LQTS is acquired, caused by medications that block HERG K^+ channels, such as certain antiarrhythmic drugs, antihistamines and antibiotics and is exacerbated by bradycardia and hypokalemia[1]. LQTS can also be inherited as an autosomal dominant or recessive disorder[2]. The more severely affected individuals can have intermittent syncope caused by torsades de pointes arrhythmia. Sudden cardiac death can occur if torsades de pointes degenerates into ventricular fibrillation.

There are 7 recognized forms of inherited LQTS[3], commonly referred to by their original loci assignment LQT1 – LQT7, and encode the genes shown in Table 1.

Table 1. Genes associated with long QT syndrome

Locus	gene	channel type (subunit)	current
LQT1	*KCNQ1*	slow delayed rectifier (α)	I_{Ks}
LQT2	*HERG*	rapid delayed rectifier (α)	I_{Kr}
LQT3	*SCN5A*	cardiac sodium (α)	I_{Na}
LQT4	unknown		
LQT5	*KCNE1*	minK (β)	I_{Ks}
LQT6	*KCNE2*	MiRP1 (β)	I_{Kr}
LQT7	*KCNJ2*	Kir2.1 inward rectifier (α)	I_{K1}

Mutations in any of the K^+ channel subunits cause a decreased outward K^+ current during the plateau phase of the cardiac action potential, delayed ventricular repolarization and an increased QT interval. LQTS caused by dominant mutations in *KCNQ1, HERG, SCN5A,* and *KCNE1* is called Romano-Ward syndrome. Recessive mutations in these genes cause Jervell and Lange-Nielsen syndrome, a more severe form of LQTS associated with neural deafness, greater prolongation of the QT interval and increased risk of sudden death. Mutations in *SCN5A* interfere with Na channel inactivation[4], a gain of function effect. Mutations in *KCNJ2* cause Andersen's syndrome, characterized by arrhythmia and periodic paralysis[5]. There are presently more than 200 known mutations associated with LQTS. Mutations in *HERG* and *KCNQ1* are common, whereas mutations in *SCN5A, KCNJ2, KCNE1*and *KCNE2* are rare. At the present rate of discovery, many more LQTS-associated mutations in these genes are likely to be described in the future. Moreover, other genes that cause LQTS (e.g., LQT4) remain to be discovered.

Mutations in the K^+ channel subunits cause loss of function or altered function of the channel, and very often a dominant-negative effect. Many missense mutations in the K^+ channel proteins cause subunit misfolding, which disrupts the co-assembly of subunits and usually leads to early degradation of the channel complex. This "dominant-negative effect"

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causes a greater than 50% reduction in the number of functional channels. A dominant-negative effect can also occur if the missense mutation results in change in an amino acid with a function vital to the operation of the channel. For example, a missense mutation of a residue located in the selectivity filter (G638S) has no effect on folding or trafficking of the HERG channel to the surface membrane[6], but causes complete loss of function. Co-expression of normal and G628S HERG subunits indicates that co-assembly of even a single G628S subunit in the tetrameric channel results in loss of function, a "lethal" dominant-negative effect[7].

Other missense mutations result in subunits that can still co-assemble with normal subunits, but alter one or properties of the channel. For example, specific mutations in *HERG* have been shown to shift the voltage dependence of gating associated with either activation or inactivation [8]. Either a shift in the voltage dependence of inactivation to a more negative potential, or a shift in the voltage dependence of activation to a more positive potential causes a reduction in I_{Kr} amplitude. Missense mutations in the amino terminus of HERG cause the channels to deactivate much faster than normal[9]. An increase in the rate of deactivation blunts the voltage-dependent increase in I_{Kr} that normally results from rapid recovery from inactivation and slow deactivation of channels during repolarization. Missense mutations in *KCNE1* (e.g., S74L, D76N) have also been shown to cause an increase in the rate of I_{Ks} deactivation and a reduction in magnitude[10]. In all cases, these LQTS-associated mutations result in a decrease in the magnitude of delayed rectifier K^+ currents I_{Ks} or I_{Kr} during the repolarization phase of ventricular action potentials, and the associated prolongation of the QT interval on the ECG.

Acquired long QT syndrome

Many commonly used medications, including antiarrhythmic, antihistamine, antipsychotic and antibiotic agents are associated with acquired LQTS. The mechanism(s) responsible for drug-induced LQTS has not been definitively determined for all these agents, but for those that have been studied, a common effect has been identified. These drugs either block I_{Kr} or inhibit liver enzymes that are important for metabolic degradation of other drugs that block I_{Kr}. Thus, drug-induced LQTS is mechanistically linked to LQT2 caused by mutations in HERG. While the factors that determine which patients are at greatest risk for developing drug-induced LQTS and arrhythmia are not fully understood, low serum K^+ or Mg^{2+}, congenital LQTS and polymorphisms in the known LQTS genes are important risk factors[11].

The most extensively characterized I_{Kr} blockers are the methanesulfonanilide class III antiarrhythmic agents (e.g., MK-499, dofetilide). These compounds were developed to prevent ventricular fibrillation, however, these drugs also have the unfortunate side-effect of inducing ventricular arrhythmias. Most HERG channel blockers bind to specific residues of the S6 domain (e.g., Y652, F656) that line the central cavity of the channel[12]. The HERG channel is unusual in having these aromatic residues in S6 and also differs from most other Kv channels by not having Pro residues in S6. These structural features contribute to the sensitivity of HERG channels to drugs. Access to this site by drug requires channel opening. Once inside the channel vestibule, closure of the activation gate can trap some drugs (e.g., MK499) inside the channel and greatly slows recovery of the channel from the blocked state[13]. Because drug-induced LQTS is such a common observation, most new drugs are now tested for HERG channel block activity.

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References:

1. Roden, D.M. Mechanisms and management of proarrhythmia. *Am. J. Cardiol.* **82**, 49I-57I (1998).
2. Schwartz, P.J., Locati, E.H., Napolitano, C. & Priori, S.G. *Chapter 72: The long QT syndrome*, 788-811 (W.B. Saunders Co., Philadelphia, 1995).
3. Keating, M.T. & Sanguinetti, M.C. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* **104**, 569-80. (2001).
4. Bennett, P.B., Yazawa, K., Makita, N. & George, A.L. Molecular mechanism for an inherited cardiac arrhythmia. *Nature* **376**, 683-685 (1995).
5. Plaster, N.M. *et al.* Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* **105**, 511-9. (2001).
6. Zhou, Z., Gong, Q., Epstein, M.L. & January, C.T. HERG channel dysfunction in human long QT syndrome. Intracellular transport and functional defects. *J. Biol. Chem.* **273**, 21061-6 (1998).
7. Sanguinetti, M.C., Curran, M.E., Spector, P.S. & Keating, M.T. Spectrum of HERG K^+ channel dysfunction in an inherited cardiac arrhythmia. *Proc. Natl. Acad. Sci. USA* **93**, 2208-2212 (1996).
8. Nakajima, T. *et al.* Novel mechanism of HERG current suppression in LQT2: shift in voltage dependence of HERG inactivation. *Circ. Res.* **83**, 415-22 (1998).
9. Chen, J., Zou, A., Splawski, I., Keating, M.T. & Sanguinetti, M.C. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. *J. Biol. Chem.* **274**, 10113-8 (1999).
10. Splawski, I., Tristani-Firouzi, M., Lehmann, M.H., Sanguinetti, M.C. & Keating, M.T. Mutations in the *hminK* gene cause long QT syndrome and suppress I_{Ks} function. *Nature Genetics* **17**, 338-340 (1997).
11. Roden, D.M. *et al.* Multiple mechanisms in the long QT syndrome: current knowledge, gaps, and future directions. *Circulation* **94**, 1996-2012 (1996).
12. Mitcheson, J.S., Chen, J., Lin, M., Culberson, C. & Sanguinetti, M.C. A structural basis for drug-induced long QT syndrome. *Proc. Natl. Acad. Sci. USA* (2000).
13. Mitcheson, J.S., Chen, J. & Sanguinetti, M.C. Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. *J. Gen. Physiol.* **115**, 229-240 (2000).

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Pathological implications of the acetylcholine receptor (AChR) channelopathies

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Mutations in AChR subunit genes cause congenital myasthenic syndromes (CMS). The mutations have clinical, morphologic, and electrophysiologic implications. Moreover, investigation of the AChR channelopathies (1) highlights functionally significant domains of the AChR, (2) yields precise structure-function correlations for synaptic transmission, (3) complements knowledge gained from site-directed mutagenesis, and (4) is directly relevant to therapy. The AChR mutations either increase or decrease the synaptic response to ACh. An increased synaptic response occurs in the slow-channel syndromes. Here mutations in different domains of different AChR subunits prolong the duration of channel opening events by increasing affinity for ACh, or gating efficiency, or both. The prolonged synaptic currents result in depolarization block on physiologic activity as well as cationic overloading of the postsynaptic region that leads to an endplate myopathy with loss of AChR. These CMS respond well to long-lived open channel blockers of AChR, such as quinidine or fluoxetine. A decreased synaptic response occurs in the (1) fast channel syndromes and (2) with low expressor AChR mutations. In the fast channel syndromes different domains of different AChR subunits curtail the duration of synaptic currents by diminishing affinity, gating efficiency, or gating stability, but leave no anatomic footprint. Low-expressor AChR mutations can occur in any AChR subunit but are concentrated in the ε subunit, at least partly because persistent expression of a substituting fetal γ subunit. These CMS respond well to 3,4-diaminopyridine plus which enhances the number of quanta released by nerve impulse together with acetylcholinesterase inhibitors which increase the number of receptors activated by each quantum. The presentation will also review CMS not due mutations in AChR subunits.

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Neuromotor disorders resulting from impaired glycinergic neurotransmission: lessons from human patients and mutant mice

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Hereditary startle disease (hyperekplexia) is a rare neuromotor disorder whose symptoms can vary widely from exaggerated startle reactions to the life-threatening stiff baby syndrome. Molecular analysis has shown that many affected families have point mutations in the ligand-binding α1 subunit of the inhibitory glycine receptor, a membrane protein that controls postsynaptic inhibition of sensory and motor neurons in spinal cord, brain stem and other regions of the CNS. Different aspects of the disease phenotype are mimicked in the natural mouse mutants *spastic, spasmodic* and *oscillator,* which carry mutations in glycine receptor α and β subunits genes. Here, we report on different novel mouse mutants that also exibit severe motor symptoms. First, inactivation of the gene encoding the glycine and $GABA_A$ receptor anchoring protein gephyrin causes early postnatal death, presumably because inhibitory receptors are not localized at synaptic sites. Due to a dual function of gepyhrin as a receptor anchoring and molybdenum cofactor (Moco) synthesizing enzyme, the mice also display symptoms of hereditary Moco deficiency. Consistent with these mouse results, a point mutation in the human gephyrin gene has recently been shown to cause Moco deficiency in a patient familiy. Second, a point mutation abolishing the potentiation of α1 subunit GlyR currents by Zn^{2+} is shown to produce a late-onset myoclonic phenotype. These results underline the importance of glycine receptor modulation and synaptic localization and identify postsynaptic structural components as potential sites of disease mutations.

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Critical role of the delayed rectifier potassium channels in oxidant-induced neuronal apoptosis

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Oxidant-induced neuronal apoptosis has been shown to involve potassium and zinc dysregulation, energetic dysfunction, activation of stress-related kinases, and caspase cleavage. The temporal ordering and interdependence of these events was investigated in primary neuronal cultures exposed to the sulfhydryl oxidizing agent 2,2'-dithiodipyridine (DTDP) or the peroxynitrite donor SIN-1, compounds that induces the intracellular release of zinc. We previously observed that tetraethylammonium (TEA), high extracellular potassium, or cysteine protease inhibitors block apoptosis induced by DTDP (Aizenman et al., J. Neurochem. 75:1878; 2000). We report that both p38 and ERK phosphorylation are evident in neuronal cultures within 2 hours of a brief exposure to 100 mM DTDP. However, only p38 inhibition is capable of blocking oxidant induced toxicity. Cyclohexamide or actinomycin D do not attenuate DTDP induced cell death, suggesting that post-translational modification of existing targets, rather than transcriptional activation, are responsible for p38's deleterious effects. Indeed, an early robust increase in TEA-sensitive potassium channel currents induced by DTDP or SIN-1 is attenuated by p38 inhibition but not by caspase inhibition. Moreover, we found that activation of p38 is required for caspase 3 and 9 cleavage, suggesting that potassium currents enhancement is required for caspase activation. Based on these findings, we conclude that oxidation of sulfhydryl groups on intracellular targets results in intracellular zinc release, p38 phosphorylation, enhancement of potassium currents, caspase cleavage, and translationally independent apoptotic cell death (McLaughlin et al., J. Neurosci. 21:3303; 2001).

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Calcium channels expression at the neuromuscular junction of P/Q deficient mice. Their role in transmitter release and vesicle recycling

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Genetic analyses have revealed an important association of the gene encoding the P/Q-type voltage dependent Ca channel a1A subunit with hereditary neurological disorders. The generation of a1A-null mutant mice allows a critical examination of what features of neurotransmission depend on P/Q-type channels. The mutant mice showed a rapidly progressive neurological deficit with muscle weakness and ataxia, before dying 3-4 weeks after birth. Electrophysiological studies at the neuromuscular junction (NMJ) have shown that basic features of transmission are retained and mediated by N and R type channels, but with significant changes in the relationship between Ca2+ entry and quantal release and short-term plasticity (Urbano et al, Biophys. J. 80: 940, 2001).

We have studied synaptic vesicle recycling by stimulating the nerve terminals at 20 Hz in the presence or absence of FM dyes. Synaptic area and vesicle pool were smaller in mutant NMJs but fluorescence density was similar to the wild type. However, the kinetics of destaining was slower in the mutant mice in agreement with the low quantal content of release. P/Q channel blocker ω-Agatoxin IVA strongly inhibits distaining in wild type but was ineffective in the mutant NMJs. In contrast ω-Conotoxin and SNX-482 strongly inhibited destaining in the mutant but not in the wild type confirming that N and R- type channels mediate transmitter release in the P/Q deficient NMJs. Nitrendipine an L- type channel antagonist exerts a small reduction in distaining kinetics in both types of NMJs.

The absence of a1A subunits and the presence of the N type a1B subunits at the mutant NMJs were confirmed with immunostaining techniques. Interestingly, L-type a1D subunits were highly expressed at the mutant NMJ in spite of the limited effect of the nitrendipine on transmitter release, loading and destaining. Our experiments demonstrate the idea that P/Q, N and R-type channels all share the intrinsic capability of triggering neurotransmitter release but with differences that lead to alterations in neuromuscular transmission. Similar dysfunction at central nervous system synapses may contribute to the generation of neurological syndromes like ataxia, migraine and epilepsy.

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Session 4: Non-neuronal channels
Chair: Jeffrey L. Noebels

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Cystic fibrosis transmembrane conductance regulator function

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Cystic fibrosis is caused by mutations in the gene encoding the CFTR Cl^- channel. CFTR is a member of the ATP-binding cassette (ABC) transporter family and it contains the features characteristic of this family: two nucleotide-binding domains and two membrane-spanning domains. In addition, CFTR contains the R domain, a unique sequence not found in other ABC transporters or any other proteins. The R domain serves as the major physiologic regulator of the CFTR Cl^- channel. Upon elevation of cAMP levels, cAMP-dependent protein kinase (PKA) phosphorylates the R domain allowing ATP to open and close the channel. Yet how phosphorylation activates the channel is not well understood. Some models propose that the R domain prevents the channel from opening and that phosphorylation relieves this inhibition. Other models suggest that phosphorylation of the R domain stimulates activity. Our recent data using studies of channel function as well as proteins and peptides from the R domain suggest a model in which the R domain is predominantly random coil. This conclusion may explain the seemingly complex way in which phosphorylation regulates CFTR channel activity.

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Intracellular chloride channels: Lessons from KO mice and human disease

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Chloride conductances provide an electrical shunt that is needed for the efficient operation of the H^+-ATPase that acidifies vesicles of the endocytotic and secretory pathways. However, the molecular identities of the underlying Cl^- channels have remained obscure until recently.

There are nine different CLC Cl^- channels in mammals (1). While the first branch of this gene family encodes plasma membrane channels, it is now clear that channels belonging to the other two branches reside primarily in intracellular organelles. This was first recognised for ClC-5 (3), a channel which is mutated in Dent's disease (2), a disorder characterised by low molecular weight proteinuria and kidney stones. ClC-5 resides in endosomes of the proximal tubule (PT), where it co-localises with the H^+-ATPase and endocytosed proteins (3). This suggested a role in the acidification of the endocytotic pathway. Disrupting ClC-5 in mice affects both fluid-phase and receptor-mediated endocytosis, as well as the endocytotic retrieval of certain plasma membrane proteins in the PT (4). As the PT endocytoses hormones such as PTH and 25(OH)VitD$_3$, this leads to changes in calciotropic hormone levels and to secondary changes in the renal handling of phosphate and calcium. Thus, the vesicular ClC-5 Cl^- channel is crucial for endocytosis.

We have also disrupted the highly homologous ClC-3 Cl^- channel that is expressed in brain and several other organs (5). This led to a nearly complete degeneration of the hippocampus and photoreceptors. ClC-3 was localised to late endosomes and synaptic vesicles, to whose acidification it contributes. The degeneration of the hippocampus may be due to an altered filling of synaptic vesicles (which depends on the electrochemical H^+ gradient), or to altered intracellular trafficking (6).

Finally, we have disrupted ClC-7, a broadly expressed member of the third branch of the CLC family (7). This led to severe osteopetrosis, which is due to a failure of osteoclasts to acidify the resorption lacuna. ClC-7 is normally present in late endosomal to lysosomal compartments, but is inserted together with the H^+-ATPase into the osteoclast ruffled border upon attachment to bone. Stimulated by this finding, we also demonstrated that human patients with severe osteopetrosis have mutations in either the ClC-7 Cl^- channel, or in a subunit of the H^+-ATPase. In addition to osteopetrosis, mice with a disrupted ClC-7 gene also display retinal and CNS degeneration.

References:

(1) Jentsch, T.J., Stein V., Weinreich F., Zdebik A.A. (2002) Molecular structure and physiological function of chloride channels. *Physiol. Rev.*, in press.

(2) Lloyd S.E., Pearce S.H.S., Fisher S.E., Steinmeyer K., Schwappach B., Scheinman S.S., Harding B., Bolino M., Devoto M., Goodyer P., Rigden S.P.A., Wrong O., Jentsch T.J., Craig I.W., Thakker R.V. (1996). A common molecular basis for three inherited kidney stone diseases. Nature 379, 445-449.

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(3) Günther W., Lüchow A., Cluzeaud F., Vandewalle A., Jentsch T.J. (1998) ClC-5, the chloride channel mutated in Dent's disease, co-localizes with the proton pump in endocytotically active kidney cells. *Proc. Natl. Acad. Sci. U.S.A. 95*, 8075-8080.

(4) Piwon N., Günther W., Schwake M., Bösl M.R., Jentsch T.J. (2000) ClC-5 Cl--channel disruption impairs endocytosis in a mouse model for Dent's disease. *Nature* 408, 369-373.

(5) Stobrawa S.M., Breiderhoff T., Takamori S., Engel D., Schweizer M., Zdebik A.A., Bösl M.R., Ruether K., Jahn H., Draguhn A., Jahn R., Jentsch T.J. (2001) Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron* 29, 185-196.

(6) Kornak U., Kasper D., Bösl M.R., Kaiser E., Schweizer M., Schulz A., Friedrich W., Delling G., Jentsch T.J. (2001) Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell* 104, 205-215.

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Regulation of the epithelial sodium channel (ENaC) by intra- and extra-cellular signaling pathways: role in salt sensitive hypertension

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According to the hypothesis put forward by Guyton, over 20 years ago [1], control of blood pressure at steady state and on a long-term basis is critically dependent on renal mechanisms. In the Aldosterone-Sensitive Distal Nephron (ASDN), the final control of sodium reabsorption is achieved through an amiloride-sensitive electrogenic sodium reabsorption which is under tight hormonal control, aldosterone playing the key role[2]. The main limiting factor in sodium reabsorption in this part of the nephron is the apically located amiloride-sensitive epithelial sodium channel (ENaC) [3]. Two monogenic diseases have been linked to ENaC subunit genes; first, pseudohypoaldosteronism Type 1, a severe autosomal recessive form of a salt-loosing syndrome is due to loss (or partial loss) of function mutations in the α, β or γ subunit genes of ENaC [4]. Gain of function mutations in the β or γ subunit of ENaC lead to a hypertensive phenotype (Liddle syndrome), a paradigm for salt-sensitive hypertension [5]. The classic model of the mechanism of aldosterone action in proposes the following steps: aldosterone crosses the plasma membrane and binds to its cytosolic receptor, either the mineralocorticoid (MR) or glucocorticoid (GR) receptor. MR and GR are protected from illicit occupation by high levels of plasma glucocorticoids (cortisol or corticosterone), thanks to the metabolizing action of 11-β-HSD2 that transforms the active cortisol into cortisone, an inactive metabolite, unable to bind either to MR or GR. The receptor-hormone complex is translocated to the nucleus where it interacts with the promoter region of target genes activating or repressing their transcriptional activity. Induced or repressed proteins AIPs or ARPs mediate an increase in transepithelial sodium transport. Early effects are produced by the activation of pre-existing transport proteins (ENaC, Na,K-ATPase). Components of the aldosterone-dependent signaling pathway have recently been identified. Aldosterone induced rapidly the expression of Sgk1 kinase (serum and glucocorticoid regulated kinase) a member of the PKB-AKT family of serine-threonine kinases[6]. When coexpressed in *Xenopus laevis* oocytes, Sgk1 stimulates ENaC activity by 2-3 fold[7,8]. Sgk1 increases cell surface expression of ENaC without changing its open probability[9,10]. Recently, it has been demonstrated that the phosphorylation of Nedd4-2, an ubiquitin protein ligase, by Sgk1 may regulate epithelial sodium channel cell surface expression in the *Xenopus* oocyte system[11]. It was further demonstrated that the phosphorylation of Nedd4-2 decreases its affinity for ENaC, thereby diminishing ENaC endocytosis and/or degradation[11,12]. These data provided evidence for the missing link between aldosterone binding to its receptor, transcription activation of an aldosterone-induced protein (Sgk1) and the sodium transport response thereby defining an *aldosterone-dependent intracellular signalling pathway* for ENaC activation.

We have identified a membrane-bound serine protease that acts as channel activating protease, namely CAP1 [13-15]. This regulation defines a novel membrane-bound *extracellular signaling pathway*, which appears to be highly conserved throughout evolution. The mechanism by which serine proteases like CAP1 activates ENaC is not yet understood. CAP1 leads to a very substantial increase in the open probability of ENaC, whereas the number (N)

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of channels at the cell surface is either unchanged[13] or even diminished[15]. We have recently identified two additional membrane-bound serine proteases (CAP2 and CAP3). We showed that each of these proteins is able to induce a 5-10 fold increase of ENaC activity in the *Xenopus* oocyte expression system by an increase of Po. The serum- and glucocorticoid-regulated kinase (Sgk1) alone enhanced ENaC activity by two fold by increasing the number of ENaC molecules at the cell surface without changing Po. Coexpression of Sgk1 with CAP1 (or CAP2 or CAP3) leads to an up to 30 fold increase in ENaC activity by increasing NxPo dramatically. The synergistic effect of the two pathways allows a large dynamic range for ENaC-mediated sodium regulation crucial for the control of bood volume and blood pressure.

References:

1. Guyton, A. C. Blood pressure control - Special role of the kidneys and body fluid. *Science* **252**, 1813-1816 (1991).
2. Verrey, F., Hummler, E., Schild, L. & Rossier, B. C. in *The Kidney, Physiology and Physiopathology* (ed. Giebisch, D. W. S. a. G.) 1441-1471 (Lippincott Williams & Wilkins, Philadelphia, 2000).
3. Garty, H. & Palmer, L. Epithelial sodium channels: function, structure, and regulation. *Physiol.Rev.* **77**, 359-396 (1997).
4. Chang, S. S. et al. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nature Genetics* **12**, 248-53 (1996).
5. Shimkets, R. A. et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* **79**, 407-14 (1994).
6. Webster, M. K., Goya, L., Ge, Y., Maiyar, A. C. & Firestone, G. L. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Molecular & Cellular Biology* **13**, 2031-40 (1993).
7. Chen, S. Y. et al. Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proceedings of the National Academy of Sciences of the United States of America.* **96**, 2514-2519 (1999).
8. Naray-Fejes-Toth, A., Canessa, C., Cleaveland, E. S., Aldrich, G. & Fejes-Toth, G. sgk is an aldosterone-induced kinase in the renal collecting duct - Effects on epithelial Na+ channels. *Journal of Biological Chemistry.* **274**, 16973-16978 (1999).
9. Alvarez de la Rosa, D., Zhang, P., Naray-Fejes-Toth, A., Fejes-Toth, G. & Canessa, C. M. The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of Xenopus oocytes. *The Journal of Biological Chemistry* **274**, 37834-37839 (1999).
10. Loffing, J. et al. Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: possible role of SGK. *American Journal Of Physiology Renal Physiology* **280**, F675-F682 (2001).
11. Debonneville, C. et al. Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na+ channel cell surface expression. *EMBO J* **20**, 7052-7059 (2001).
12. Snyder, P. M., Olson, D. R. & Thomas, B. C. SGK modulates Nedd4-2-mediated inhibition of ENaC. *J.Biol.Chem.* **277**, 5-8 (2002).
13. Vallet, V., Chraibi, A., Gaeggeler, H. P., Horisberger, J. D. & Rossier, B. C. An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature* **389**, 607-10 (1997).
14. Vallet, V., Horisberger, J. D. & Rossier, B. C. Epithelial sodium channel regulatory proteins identified by functional expression cloning. *Kidney International* **54**, S109-S114 (1998).
15. Vuagniaux, G. et al. Activation of the amiloride-sensitive epithelial sodium channel by the serine protease mCAP1 expressed in a mouse cortical collecting duct cell line. *Journal of the American Society of Nephrology* **11**, 828-34 (2000).

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The EAG potassium channel is involved in cancer

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Potassium channels are implicated in rapid signaling processes. Their high variability and their ubiquity indicate that they might be also implicated in many other functions. We have described a voltage-operated, potassium selective channel (EAG) that is under strict control during the cell cycle. One of the most characteristic properties of the channel, its ionic selectivity, is modulated during the G2/M transition. Strikingly, the expression of the human EAG is restricted to brain, but it is also present in several tumor-derived cell lines. In vitro experiments allowed us to conclude that the channel is not only influenced by the cell cycle, but its overexpression can change the proliferation properties of the cells. EAG-transfected cells grow faster than controls. They also lose contact inhibition, as well as growth factor-dependence and substrate attachment requirement. In summary, EAG-transfected cells show a transformed phenotype. Moreover, inhibition of the channel in human tumor cell lines leads to a reduction in DNA synthesis and proliferation. To validate these results obtained in vitro on primary tumors, we generated monoclonal antibodies against the channel protein and used these for immunohistochemical studies. Whenever possible, we correlated these results with expression studies using quantitative real-time PCR.

We concentrated on primary breast carcinomas, because we had found the channel in ductal carcinoma-derived cell lines. While normal breast tissue is not stained with our antibodies, over 80% of the tumors tested showed strongly positive staining. The ectopic expression of EAG in many tumor tissues was confirmed by RT-PCR.

Not only mammary carcinoma was found to be positive for EAG, but also other solid tumors showed clearly positive signals with high frequency. Lung, prostate, colon and liver carcinoma are positive in more than 80% of the cases studied.

We conclude that EAG is a widely distributed tumor marker. It offers the possibility for the design of a new therapeutic approach, since it seems to be required for tumor cell growth. It is accessible from the outside in intact cells, apparently present in very low amounts in normal cells although abundant in tumor cells, its normal location being protected by the blood-brain barrier.

References:

L.A. Pardo, A. Brüggemann, J. Camacho and W. Stühmer: Cell-cycle related changes in the conducting properties of r-EAG K+ channels. J. Cell. Biol. 143, 767-775 (1998)

L.A. Pardo, D. del Camino, A. Sánchez, F. Alves, A. Brüggemann, S. Beckh and W. Stühmer: Oncogenic potential of EAG K+ channels. EMBO J. 18, 5540-5547 (1999).

J. Camacho, A. Sánchez, W. Stühmer and L.A. Pardo: Cytoskeletal interactions determine the electrophysiological properties of human EAG potassium channels. Pflügers Arch. – Eur. J. Physiol. 441, 167-174 (2000).

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ATP-sensitive K-channels: regulation and roles in health and disease

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ATP sensitive K-channels (K_{ATP} channels) play important roles in a diverse range of tissues (including pancreatic β̃cells, neurones, and cardiac, skeletal and smooth muscles) by coupling the metabolic state of the cell to its electrical activity (Ashcroft and Gribble, 1999). In pancreatic β-cells, K_{ATP} closure in response to glucose metabolism produces membrane depolarization, leading to Ca^{2+} influx and insulin secretion. K_{ATP} channels are also involved in neuronal glucose sensing, glucose uptake in skeletal muscle, seizure protection, regulation of vascular tone and the response to cardiac and cerebral ischemia. Metabolic regulation is mediated by changes in intracellular ATP (which blocks the channel) and MgADP (which activates the channel). K_{ATP} channels are also inhibited by sulphonylurea drugs, which stimulate insulin secretion and are used to treat type 2 diabetes, and activated by K_{ATP}-channel openers, a structurally diverse group of drugs with a wide range of potential therapeutic applications.

The β-cell K_{ATP} channel is 4:4 heteromeric complex of pore-forming Kir6.2 and regulatory SUR1 subunits. SUR1 endows the channel with sensitivity to the stimulatory effects of MgADP and K_{ATP}-channel openers and the inhibitory action of sulphonylureas. ATP closes the channel by binding to Kir6.2. Although mutation of both N and C terminal residues of Kir6.2 reduces ATP inhibition (Tucker et al., 1988), it is not clear whether the nucleotide interacts directly with these residues or if the reduction in ATP sensitivity is mediated allosterically. To determine which residues contribute directly to the ATP-binding site, we used a cysteine substitution approach, in combination with thiol modification. Thiol reagents bind irreversibly to cysteine residues and provide a means to introduce either a positive (MTSEA) or negative (MTSES) charge into the protein. If the ATP-binding site lies sufficiently close to the modified cysteine, the introduced charge will exert an electrostatic effect on ATP binding and thus on the ability of ATP to close the channel. Comparison of the ATP sensitivity of cysteine-mutant channels (expressed in *Xenopus* oocytes) before and after thiol modification revealed that the side-chains of several residues (eg. R50, K185) lie within a few angstroms of the phosphate tail of the bound ATP molecule. Thus, the ATP-binding site contains contributions from both the amino (R50) and carboxy (K185) termini of Kir6.2.

Binding of ATP to Kir6.2 subunit is allosterically linked to closure of the channel pore. To elucidate the location of the conformational changes associated with channel closing, we examined the block by Ba^{2+} ions. The on-rate of Ba^{2+} block was not slowed by the presence of ATP, as would be expected if access of Ba^{2+} to its blocking site is restricted when the channel is shut. Thus the gate closed by ATP lies above the Ba^{2+} binding site, which lies at the inner end of the selectivity filter. This is in marked contrast to voltage-gated K^+ channels, where the helix bundle crossing at the inner mouth of the K_{ATP} channel serves as a gate for K^+ ions.

The K_{ATP} channel plays a key role in the regulation of insulin secretion in both health and disease. Mutations in SUR1 or Kir6.2 that result in channel closure produce congenital

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hypoglycaemia of infancy in man, a disease of excessive insulin secretion (Aguilar-Bryan and Bryan, 1999). Conversely, impaired cell metabolism, or mutations in Kir6.2, which lead to enhanced channel activity, result in diabetes. Interestingly, a new SUR1 variant (E1506K) that causes congenital hyperinsulinism in infancy, and loss of insulin secretory capacity in early adult life, also leads to diabetes in middle-age. This variant of the SUR1 gene represents a new subtype of diabetes, fulfilling the criteria for an autosomal dominant form of diabetes.

References:

Aguilar-Bryan and Bryan (1999) Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Reviews* **20**, 101-135

Ashcroft FM, Gribble FM (1999) ATP-sensitive K^+ channels in health and disease. *Diabetologia* **42**, 903-919.

Proks P, Capener C, Jones P, Ashcroft FM (2001). Mutations within the P-loop of Kir6.2 modulate the intraburst kinetics of the ATP-sensitive potassium channel. *Journal of General Physiology* **118**, 341-353

Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T, Reimann F, Ashcroft FM (1998) Molecular determinants of K_{ATP} channel inhibition by ATP. *EMBO Journal* **17**, 3290-3296.

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Session 5: Other channels
Chair: Nathaniel Heintz

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Role of vanilloid receptor I in inflammatory pain

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Identification and cloning of the VR1 channel represented a significant step in the clarification of the molecular mechanisms underlying transduction of noxious chemical and thermal stimuli by peripheral nociceptors. This receptor is a nonselective cation channel with high Ca^{2+} permeability activated by capsaicin and its analogues, as well as by endocannabinoids. In addition, VR1 is gated also by noxious heat (>43°C) and low pH. There is evidence that VR1 plays a key role in both nociception and inflammatory pain. For instance, VR1 channel activity is strongly up regulated by the action of inflammatory mediators such as NGF, bradykinin and prostaglandins, presumably by PKC or PKA mediated receptor phosphorylation. Accordingly, VR1 has been considered a molecular integrator of noxious stimuli in the peripheral terminals of primary sensory neurons involved in pain signaling. Although there are no reported VR1 gene mutations as causative of human disease, the possibility that pain sensitivity and/or certain painful pathologies have a genetic basis is being considered. Valuable information on the physiological and pathological role of the VR1 channel has been gained from genetic and pharmacological knock-out of VR1 channels in mice. These studies highlight a major role of VR1 in the onset of nociceptive responses to heat and in primary hyperalgesia elicited by tissue damage, thus underscoring its interest as a therapeutic target for treating specific types of pain. The presentation will also address the role of other ion channels in the pathophysiology of pain transduction.

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Biological functions of connexin channels analyzed with targeted mouse mutants

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Gap junctions are clustered channels between contacting cells through which direct intercellular communication via diffusion of ions and metabolites can occur. Two hemichannels, each built up of six connexin protein subunits in the plasma membrane of adjacent cells, can dock to each other to form conduits between cells. We have recently screened mouse and human genomic data bases and have found 19 connexin genes in the mouse genome and 20 connexin genes in the human genome (Willecke et al., 2002). Targeted ablation of 11 mouse connexin genes and the phenotypes of human genetic disorders caused by mutated connexin genes reveal basic insights into the functional diversity of the connexin gene family.

Here I shall first discuss recent results from our laboratory concerning cardiomyocyte specific or induced deletion of connexin43 which lead to sudden death due to arrhythmias. Previously it had been shown that the general deletion of the connexin43 gene led to death shortly after birth due to obstruction of the right ventricular outflow tract of the heart. Connexin43 is expressed in several cell types, most abundantly in cardiomyocytes and astrocytes. Astrocyte specific deletion of connexin43 was accomplished after crossing floxed connexin43 mice with GFAP (glial fibrillary acid protein) promoter Cre mice. Under these conditions, the loss of connexin43 could be monitored in a cell autonomous manner via conditional replacement of the connexin43 coding region by a LacZ reporter gene. Surprisingly, these mice showed increased velocity of hippocampal spreading depression (collaboration with Regina Jauch and Uwe Heinemann, Berlin, Germany) in the *stratum radiatum*. Spreading depression is a wave of depolarization followed by neuronal inactivation that was triggered by pressure injection of potassium into the CA1 region.

After general deletion of the connexin36 gene which is expressed in neurons, we observed in transgenic mice that the electroretinogram as well as the vision evoked potentials in the optic tectum were altered, suggesting functional synchronization of retinal amacrin II cells during light reception (Güldenagel et al., 2001). Finally, we have recently found, together with the group of Christine Petit, Institut Pasteur, France, that mice with cell type specific deletion of the connexin26 gene in cochlear supporting cells suffer from severe hearing impairment. The analytical results and our hypothesis to explain the mechanism of this channelopathy will be discussed.

References:

Willecke, K., Eiberger, J., Degen, J., Eckardt D., Romualdi, A., Güldenagel, M., Deutsch, U., and Söhl, G. 2002. Structural and functional diversity of connexin genes in the human and mouse genome. Biol. Chem., in press.

Güldenagel, M., Ammermüller, J., Feigenspan, A., Teubner, B., Degen, J., Söhl, G., Willecke, K., Weiler, R. 2001. Visual transmission deficits in mice with targeted disruption of the gap junction gene connexin36. J. Neuroscience 21: 6034-6044.

Cohen-Salmon, M., Ott, O., Michel, V., Hardelin, J.-P., Perfettini, I., Eybalin, M., Wu, T., Marcus, D.C., Wangemann, P., Willecke, K., and Petit, C. 2002. Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and massive cell apoptosis, submitted for publication.

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Functional analysis of human mutations associated with connexinophaties

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An increasing number of human diseases are being associated with mutations in the genes of connexins (Cx), the family of proteins forming gap junction channels in vertebrates, whose phenotype reflects the inferred function of the affected genes. To date, more than 260 different mutations of Cx32 gene have been identified in the X-linked form of Charcot-Marie-Tooth disease (CMTX) with variable clinical severity. Mutations in Cx26 are the most frequent cause of congenital deafness while certain mutations of Cx26, Cx31 and Cx30 have been found in families with dominant progressive hearing loss. Autosomal dominant mutations in four connexins (Cx26, Cx31, 30.3 and Cx30) have been demonstrated in inherited skin disorders with or without hearing impairment. In addition, mutations in Cx50 and Cx46 genes have been linked to certain forms of inherited congenital cataract. Most of mutations occur into the coding region and they are distributed throughout all topological domains of connexin molecules. Attempts to explain from the location and type of mutation (deletion or insertion, and nonsense or missense substitution) the molecular and cellular mechanisms leading to the phenotype have been hampered by our limited knowledge concerning the molecule structure and contribution of the various molecular domains to the formation of channels or channel function. We have explored the functional consequences of Cx mutations using an *in vitro* expression system composed of paired *Xenopus* oocytes. Before pairing, oocytes can be injected with a single Cx RNA species, or with a mixture of two Cx RNAs, so that either recessive mutations, or interactions between dominant mutants and wild-type connexins can be studied. The main conclusion arising from these studies is that different connexin mutations cause disease by several pathomechanims. Many of mutations induced a complete loss of function to form the gap junction channels whereas others reduced the efficiency of channel formation (1). Mutations might also alter permeation or gating properties (2). In addition, we show an unusually severe form of peripheral neuropathy due to a new Cx32 mutation with a novel gain-of-function effect, which can affect the cellular viability. Other connexin diseases are dominantly inherited, consistent with a dominant or toxic effect of the mutations.

References:

(1) Castro, C, *et al,* (1999). *J. Neurosci.* 19:3752-3760.
(2) Revilla, A, *et al,* (1999). *Biophys. J.* 77:1374-1383.

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Molecular determinants in GABAergic interneurones for network synchrony and oscillatory activity

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There is increasing evidence that GABAergic interneurones, in addition to being the main source of inhibition in the adult brain, are critically involved in the generation of synchronous activity in large networks of pyramidal neurones and of oscillatory activity. Our experimental efforts, which include the use of gene-manipulated mice, are directed towards addressing the following questions: 1. Which are the critical players regarding neurochemical transmission in GABAergic interneurones that are important for synchrony and oscillations? 2. To which extent is electrical neurotransmission via gap junctions important, in which cell types does it occur and which brain rhythms are affected? 3. Which are the GABAergic interneurone subtypes that control brain rhythms at different frequencies? Thus, the presentation will address the importance of differential AMPA receptor expression in GABAergic interneurones, the role of Cx36 expression in interneurones and the scientific potential residing in the technique of in vivo labelling of different types of GABAergic interneurones. The latter is a tremendous aid for functional and anatomical characterization of the different GABAergic interneurone subtypes in the acute brain slice but also in vivo.

References:

Venance, L., Rozov, A., Blatow, M., Burnashev, N., Feldmeyer, D., Monyer, H.: Connexin expression in electrically coupled postnatal rat brain neurons. *Proc. Natl. Acad. Sci. USA* 97: 10260-10265 (2000).

Fuchs, E. C., Doheny, H., Faulkner, H., Caputi, A., Traub, R.D., Bibbig, A., Kopell, N., Whittington, M.A., Monyer, H.: Genetically altered AMPA-type glutamate receptor kinetics in interneurons disrupt long-range synchrony of gamma oscillation. *Proc. Natl. Acad. Sci. USA* 98: 3571-3576 (2001).

Hormuzdi, S.G., Pais, I., LeBeau, F.E.N., Towers, S.K., Rozov, A., Buhl, E.H., Whittington, M.A., Monyer, H.: Impaired Electrical Signalling Disrupts Gamma Frequency Oscillations in Connexin 36 Deficient Mice. *Neuron* 31: 487-495 (2001).

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Identification of a non-continuous calmodulin binding site in non-inactivating voltage-dependent KCNQ potassium channels

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The KCNQ family of voltage-dependent potassium selective channels are involved in the control of cellular excitability. Remarkably, mutations in four of the five known members of this family have been associated with different hereditary human disorders. While mutations in the KCNQ1 subunit (KvQT1) lead to arrhythmia in the human long QT syndrome, mutations in KCNQ2 or KCNQ3 are associated with a benign form of epilepsy (BFNC). It has also been shown that KCNQ4 is mutated in a dominant form of progressive hearing loss.

KCNQ2 and KCNQ3 subunits have been shown to form M-type potassium channels. The M-current (I_M) is a subthreshold non-inactivating voltage-dependent potassium current that is modulated by a variety of intracellular signals that, in turn, dramatically effect the firing rate of neurons. Among those intracellular signals, the inhibition of I_M by B_2 bradykinin receptors in sympathetic neurons has been shown to be mediated by Ca^{2+}. Indeed, intracellular Ca^{2+} can suppress M-channels under conditions that do not support enzymatic activities such as phosphorylation, suggesting that an intermediary might be involved in this Ca^{2+}-dependent modulation.

In a search for candidates that might mediate the effects of Ca^{2+} in modulating I_M, we screened a human brain cDNA library using the yeast two hybrid system. We found that calmodulin (CaM) binds to the intracellular C-terminal region of all the known members of the KCNQ family of potassium channels. Moreover, in two-hybrid assays where it is possible to detect interactions with apo-CaM but not with Ca^{2+}-CaM, we localized the CaM binding site to a region that is predicted to contain two α-helices (A and B). These two helices encompass ~85 amino acids and, in KCNQ2, they are separated by a dispensable stretch of ~130 amino acids. We hypothesize that those two helices come into close proximity in the tertiary structure, facilitating CaM binding.

Within this CaM binding domain we found an IQ-like CaM binding motif in helix A and two overlapping consensus 1-5-10 CaM binding motifs in helix B. Point mutations in helix A or B were capable of abolishing CaM binding in the two hybrid assay, and GST fusion proteins containing helices A-B bound CaM, indicating that the interaction with KCNQ channels is direct. Full-length CaM (both N- and C-lobes), and a functional EF-1 hand were required for these interactions to occur. These observations suggest that apo-CaM is bound to KCNQ channels at low resting Ca^{2+} levels, and that this interaction is disturbed when the $[Ca^{2+}]$ is raised.

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POSTERS

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Beta-adrenoceptor stimulation restores transient outward potassium current in diabetic cardiomyocytes

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Diabetic myocardiopathy reduces the amplitude of the cardiac transient outward K^+ current, Ito, and accelerates current inactivation. We have previously demonstrated that the reduction of current amplitude is related to a diminution of the trophic effect of sympathetic nervous system on the myocardial cell, due to diabetic neuropathy. Now, we try to elucidate the mechanisms involved in the trophic effect of norepinephrine on Ito current expression. Ito current was recorded using the whole-cell variation of the Patch-Camp technique. Myocytes were isolated form the right ventricle of streptozotocin-induced diabetic rats, and incubated for at least 24 hours with the different trophic factors and inhibitors. Long-lasting exposure of diabetic myocytes to norepinephrine increases current amplitude near to control values. Previous exposure of the cells to the a1-blocker prazosin, or to the b-blocker propranolol, demonstrated that the effect is due to the neurotransmitter interaction with b-adrenoceptors. A concentration response curve done with the b-agonist isoproterenol showed that the trophic effect on Ito expression is concentration dependent, with a maximum effect obtained with 1 nM. With higher concentrations the effects obtained are reduced, probably due to b-adrenoceptor desensibilisation.

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Barttin is a Cl^- channel beta-subunit crucial for renal Cl^- reabsorption and inner ear K^+ secretion

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Renal salt loss in Bartter's syndrome is caused by impaired transepithelial transport in the loop of Henle. Sodium chloride is taken up apically by the combined activity of NKCC2 (Na^+-K^--$2Cl^-$ cotransporters) and ROMK potassium channels. Chloride ions exit from the cell through basolateral ClC-Kb chloride channels. Mutations in the three corresponding genes have been identified that correspond to Bartter's syndrome types 1–3. The gene encoding the integral membrane protein barttin is mutated in a form of Bartter's syndrome that is associated with congenital deafness and renal failure. Here we show that barttin acts as an essential -subunit for ClC-Ka and ClC-Kb chloride channels, with which it colocalizes in basolateral membranes of renal tubules and of potassium-secreting epithelia of the inner ear. Disease-causing mutations in either ClC-Kb or barttin compromise currents through heteromeric channels.

Currents can be stimulated further by mutating a proline-tyrosine (PY) motif on barttin. This work describes the first known -subunit for CLC chloride channels and reveals that heteromers formed by ClC-K and barttin are crucial for renal salt reabsorption and potassium recycling in the inner ear.

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VR1 sensitization underlies inflammatory pain

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Vanilloid receptor subtype 1 (VR1) appears to play a fundamental role in the transduction of peripheral tissue injury and/or inflammation responses. This receptor is implicated in thermal and chemical nociception and has been suggeted to contribute to thermal hyperalgesia. Nociceptor sensitization with inflammatory mediators gives rise to an increase of VR1 channel activity that results from the receptor phosphorylation. We are using a chemical genomics approach to unravel the specific participation of this receptor channel in inflammatory pain. We report the identification of a family of novel N-alkylglycine based, channel blockers of this receptor that exhibit in vivo anti-nociceptive and analgesic activity. These compounds blocked capsaicin-operated ionic currents with submicromolar efficacy and weak voltage dependency. Analysis of the mechanism of action showed that the drug binding site is located at the entryway of the channel permeation pathway. These compounds attenuated capsaicin-induced Ca^{2+} influx in trigeminal nociceptors in culture, and eliminated the vanilloid stimulated nerve activity in afferent fibers. Intraperitoneal administration resulted in thermal anti-nociception, as well as significant analgesic activity against inflammatory pain elicited by irritants such as capsaicin and mustard oil. Taken together, our findings substantiate the notion that VR1 sensitization is a major component of inflammatory pain. Therefore, down regulation of sensitized VR1 channels should be considered a prime therapeutic target for pain relief.

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Diabetes-induced alterations in cardiac transient outward potassium current kinetics. Role of channel phosphorilation status

Mónica Gallego, Raúl Setién, David Fernández y Oscar Casis

Diabetic myocardiopathy reduces the amplitude of the cardiac transient outward K^+ current, Ito, and accelerates current inactivation. The reduction of current amplitude is related to a diminution of the trophic effect of sympathetic nervous system on the myocardial cell, due to diabetic neuropathy. However, the acceleration of current inactivation is due to a different and unknown mechanism. Ito channel phosphorilation by a Ca^{2+}/Calmodulin dependent kinase, CaMKII, slows current inactivation, thus, the acceleration induced by diabetes could be due to an alteration in the activity of this system. Ito current was recorded using the whole-cell variation of the Patch-Camp technique. Myocytes were isolated form the right ventricle of healthy and streptozotocin-induced diabetic rats. Different inhibitors and activators of the Ca^{2+}/Calmodulin Kinase II and phosphatase A II were added to the pipette solution. The results obtained showed that all kinase inhibitors or phosphatase activators accelerates Ito current inactivation in healthy cells, but had no effects on diabetic cells, which have current inactivation accelerated by diabetes. We concluded that Ito channel is fully phosphorilated in normal healthy myocytes, but is fully dephosphorilated in diabetic myocytes.

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The d178y mutant associated with the X-linked form of Charcot-Marie-Tooth causes multiple-dysfunctions of connexin-32 channels

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To date, more than 260 different mutations of the Cx32 have been identified in patients with the X-linked form of Charcot-Marie-Tooth disease (CMTX), a hereditary peripheral neuropathy. Mutations are distributed throughout all topological domains of the Cx32 molecule. The functional consequences of the D178Y substitution, a CMTX mutant located at the second extracellular loop, have been explored using the expression system of paired *Xenopus* oocytes. Although the D178Y mutant induced the expression of high levels of channel precursors, i.e., the hemichannels, the paired oocytes developed very low levels of electrical coupling, indicating that this mutation reduced the efficiency of hemichannels to dock and form full channels. Moreover, the D178Y mutant introduced important modifications in the unitary and gating properties of hemichannels and complete channels. The D178Y currents induced by transjunctional voltage showed a marked instantaneous rectification, which may reflect the novel rectifying properties of hemichannel at unitary level. The conductance of the D178Y junctions also exhibited novel regulatory properties by voltage. Interestingly, this mutant also altered the regulatory effects of Ca^{2+} on Cx32 hemichannels. The external Ca^{2+} exerts two types of actions on wild-type hemichannels. Ca^{2+} blocks the voltage-gated transition between the partially open state of ~18 pS (γ_{18}) and the fully open state of ~90 pS (γ_{90}) as well as it acts as a channel-blocker but only of partially open hemichannels (γ_{18}). The D178Y mutation was able to abolish these two types of blockage in presence of high concentration of Ca^{2+}. The construction of a non-related CMTX mutant, the D178N, allowed us to confirm that the residue of aspartate is necessarily involved in the binding of Ca^{2+}. This binding-site, which must be positioned near the narrowest constriction of the pore, can account for the blockages by Ca^{2+} of the pore lumen and of the voltage-gate opening as well.

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Disruption of KCC2 reveals an essential role of $K^{-}Cl^{-}$ cotransport already in early synaptic inhibition

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Synaptic inhibition is crucial for the control and modulation of neuronal activity. Disturbing the interplay between excitation and inhibition causes various neurological disorders. GABA and glycine are the main inhibitory neurotransmitters of the adult central nervous system. Synaptic inhibition by GABAA and glycine receptors, which are ligand-gated Cl^{-} channels, depends on the intracellular chloride concentration ($[Cl^{-}]i$). High $[Cl^{-}]i$ can lead to excitatory GABA responses that are deemed to be important during development. Several potassium-chloride cotransporters can lower $[Cl^{-}]i$, including the neuronal isoform KCC2, which was substantiated by antisense experiments in vitro. Analysis of the expression pattern of KCC2 during murine embryonic and postnatal development by *in situ* hybridization and Western blot analysis, shows that KCC2 parallels neuronal differentiation and precedes the functional GABA switch. Neonate KCC2 knockout (Kcc2-/-) mice die due to severe motor deficits including loss of respiration. Sciatic nerve recordings reveal abnormal spontaneous electrical activity indicating a spastic disorder. Spinal cord responses to peripheral electrical stimuli are altered in Kcc2-/-mice as observed in the mouse mutant spastic. In wild-type animals, immunofluorescence and electron microscopy demonstrated KCC2 expression close to inhibitory synapses. Patch-clamp measurements of spinal cord motoneurons demonstrated an excitatory GABA and glycine action in the absence, but not in the presence of KCC2. This shows that the functional GABA/glycine switch in the spinal cord occurs earlier than in the hippocampus. It depends crucially on the expression of KCC2, and is indispensable for the normal function of motor circuits already at birth.

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Correlation of EEG patterns and seizure phenotype in a genetic model for epilepsy

Heinz Eric Krestel

In a genetic model for epilepsy in mice, early onset epilepsy and death is caused by deficiency of RNA editing of transcripts for the AMPA receptor subunit GluR-B (GluR-2; Brusa et al, 1995). AMPA channels containing GluR-B have low Ca^{2+} permeability and low single-channel conductance. The molecular determinant in GluR-B responsible for this channel behaviour is an arginine (R) residue positioned in the channel pore. This arginine is not encoded by the GluR-B gene which carries a glutamine (Q) codon instead. The molecular mechanism underlying the switch from the glutamine codon in the gene to the arginine codon in the mRNA is termed RNA editing.

We used pharmacological and genetic tools to prevent seizures and death in young mice. Adult epileptic mice (postnatal days 50 - 70) were monitored by video and telemetry-based EEG recordings around the clock. Video tape analysis revealed that seizure attacks could be separated into periods with prevalent phenotypes such as forelimb seizures, oral automatisms, clonic and tonic seizures. These seizure phenotypes correlated with distinct EEG patterns.

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A member of the HIT protein family interacts with VR-1

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Vanilloid receptor subunit 1 (VR-1) is implicated in thermal and chemical nociception and contributes to thermal hyperalgesia. VR-1 receptors are heavily expressed in nociceptors although it has also been localized in the central nervous system. VR-1 channels appear to be homomeric receptors of unknown stoichiometry. Physiological evidence, however, hint to the existence or additional subunits or, alternatively, the specific interaction of VR-1 with cytosolic proteins that could significantly modulate its channel activity. To address this question, we have used a yeast two-hybrid screening strategy to identify proteins in a rat brain cDNA library (prey) that interact with the N-terminal domain of VR-1 (bait). Screening of 1.0 million clones resulted in the identification of the protein kinase C interacting protein I (PKCI), a member of the HIT protein family that modulates PKC activity. This is a ubiquitous protein family that contains a conserved histidine triad (His-x-His-x-His). PKCI may act as a modulator of the algogenic-induced PKC-mediated activation of VR-1 channels in inflammatory conditions.

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Molecular basis of electrophysiological diversity of neocortical interneurons

María Toledo-Rodríguez

A major challenge in the post-genomic era is to establish the functions of specific genes and combinations of genes. This becomes a high-priority issue in the case of channelopathies, pathological states suspected to be caused by ion channel malfunction. In order to determine which ion channels are causing these pathologies we must first fully understand how ion channels are express and function in non -pathological conditions. Up till now studies investigating ion channel expression and function have been limited to a few channels. Nevertheless the complex neuron's fining patterns are not result of the activity of a single ion channel but of the interaction between ion channel constellations co-expressed by the neuron. In order to study this diversity, we have undertaken a large-scale multiplex single cell RT-PCR study in which we examined the expression of over 30 ion channel alpha and beta subunits in anatomically, physiologically and molecularly characterized neurons at the single cell level. We have included virtually every channel subunit that may play a role in shaping the neurons electrophysiological behavior. These included: the voltage activated K^+ channels (Kv1.1/2/4/6, Kvb1/2, Kv2.1/2, Kv3.1/2/3/4, Kv4.1/2/3, KChIP1/2/3), calcium activated K^+ channels (SK1/2/3), K^+/Na^+ permeable hyperpolarization activated ion channels (HCN1/2/3/4) and voltage activated calcium channels (Caa1A/B/E/G/H/I, Cab1/3/4). We will present the results of detailed correlations between mRNA profiles of ion channels and the different electrophysiological features of the cell as well as correlations between expression patterns and anatomically defied neurons.

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