



Published in final edited form as:

Neurotoxicology. 2011 October ; 32(5): 661–665. doi:10.1016/j.neuro.2011.06.002.

Channelopathies: Summary of the Hot Topic Keynotes Session

Jason P. Magby¹, April P. Neal², William D. Atchison², Isaac P. Pessah³, and Timothy J. Shafer⁴

¹Joint Graduate Program in Toxicology, Rutgers University / University of Medicine and Dentistry New Jersey 170 Frelinghuysen Rd. Piscataway, NJ

²Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan

³Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA

⁴Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA

The “Hot Topic Keynotes: Channelopathies” session of the 26th International Neurotoxicology Conference brought together toxicologists studying interactions of environmental toxicants with ion channels, to review the state of the science of channelopathies and to discuss the potential for interactions between environmental exposures and channelopathies. This session presented an overview of chemicals altering ion channel function and background about different channelopathy models. It then explored the available evidence that individuals with channelopathies may or may not be more sensitive to effects of chemicals.

Dr. Tim Shafer began his presentation by defining what channelopathies are and presenting several examples of channelopathies. Channelopathies are mutations that alter the function of ion channels such that they result in clinically-definable syndromes including forms of epilepsy, migraine headache, ataxia and other neurological and cardiac syndromes (Kullmann, 2010). Because of the ubiquitous but heterogeneous nature of ion channels, channelopathy syndromes are highly variable, and depend not only on the type of channel mutated but also on how the mutation alters the function of the channel (Waxman, 2007). While there are a number of possible phenotypes resulting from channel mutations, one feature shared by a number of channelopathies is that they cause periodic and discrete attacks, allowing the carrier of the mutation to function normally between attacks (Kullmann, 2010). Like many genetic diseases, channelopathies tend to present early in life; some only cause dysfunction during development, others may continue to cause problems throughout life while others do not present overt (or at least recognized) clinical signs without environmental triggers (Pessah et al., 2010, Striessnig et al., 2010). In order to understand the full implications of these mutations, and their highly variable manifestations, it is essential to understand ion channel physiology and function. Ion channels are found in all cell types throughout the body, and have a wide variety of structures and functions.

© 2011 Elsevier B.V. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Ion channels serve as transmembrane pores that, when open, allow ions to pass across a membrane. Because movement of ions across a membrane results in electrical currents, ion channels serve essential functions in electrically excitable tissues, such as neural cells and cardiac, skeletal and smooth muscle. The opening and closing (gating) of ion channels is controlled either by changes in membrane potential (voltage (Vacher et al., 2008)), or by the binding of ligands such as neurotransmitters. Thus, ion channels typically are classified into one of two large groups based on the stimuli that activate them: voltage-gated or ligand gated.

The voltage-gated ion channel family includes voltage-gated sodium channels, voltage-gated calcium channels, and voltage-gated potassium channels. All of these channels consist of a pore-forming α subunit and associated auxiliary subunits that modify the function and/or expression of the α subunit. Mutations in either the pore forming α subunit or the auxiliary subunits can manifest as clinical syndromes. Voltage-gated sodium channels (VGSC) are responsible for neuronal depolarization as well as initiation and propagation of action potentials in the nervous system. Sodium channel mutations in humans and animals result in seizures, rare forms of familial migraine headache, movement and pain disorders (Catterall, 2000). The voltage-gated calcium channels (VGCC) are essential to cell signaling, neurotransmitter release and neuronal plasticity. Mutations in VGCC channels result in seizures, migraine, neurodegenerative disorders and myasthenic syndromes (Striessnig et al., 2010). The voltage-gated potassium channels are required for neuronal repolarization following the rising phase of the action potential. Potassium channelopathies are also associated with seizures as well as paralysis, depending on the nature of the mutation. In the heart, mutations have been described in the hERG potassium channel ($K_{v1.1}$) that disrupt its function, leading to a potentially fatal arrhythmia (*torsades de pointes*). The hERG channel has been identified as an unintended target for a number of pharmaceutical agents. In some cases block of this channel can give rise death by cardiac arrhythmia. This has made this channel particularly important in pharmaceutical safety testing.

The ligand-gated ion channel family includes nicotinic Acetylcholine receptor (nAChR), γ -aminobutyric acid receptor (GABA), glycine, and serotonin receptor (5-HT₃) channels. These channels, as their name implies, open in response to ligand binding, permitting ion flux. Each of these receptors is comprised of a homo- or heteromer of 5 receptor subunits, and each subunit has multiple isoforms. The wide variety of subunits that comprise these channels produces a wide variety of functional diversity. The most common manifestation of mutations in these ion channels is epilepsy (Kullmann, 2010). However, the specific type of epilepsy varies depending on the channel and the mutation. These range from childhood absence epilepsy, produced by a dominantly inherited mutation of the beta 3 subunit of the GABA_A receptor which reduces cell-surface expression, to autosomal dominant nocturnal frontal lobe epilepsy, caused by various mutations in nAChR subunits which result in alterations in the ratio of subunit isoforms that make up the pentameric channel and alter the channel's response to acetylcholine. Mutations in ligand-gated ion channels can also produce myasthenic syndrome as a result of a nonsense mutation producing a nAChR channel deficiency and familial hyperekplexia as a result of impaired glycine receptor function, among other phenotypes (Kullman, 2010).

One point highlighted by Dr. Shafer was that to date, most of the research on channelopathies has focused on the mutations themselves, how they alter function of the channel, and the symptoms they produce. There has been little investigation into whether mutations associated with channelopathies may also make individuals more sensitive to environmental chemicals. However, Dr. Shafer provided some specific examples that demonstrate that channelopathies make an individual more sensitive to the effects of an "environmental" agent. Mutations in potassium channels have been shown to make an

individual more susceptible to Long QT syndrome, a condition arising in the heart as a result of a defect in cardiac repolarization that can in some cases lead to *torsade de pointes*, a form of ventricular tachycardia, and death (Modell and Lehmann, 2006). In addition to the conventionally recognized triggers (pharmacological agents) able to induce or exacerbate this condition, Dr. Shafer noted that environmental agents, such as particulate matter and arsenic have been reported to interact with hERG channels. Dr. Shafer also noted that malignant hyperthermia, a potentially fatal complication associated with volatile anesthetics, is associated with mutations to the ryanodine receptor, an intracellular ion channel. Individuals that are susceptible to this complication live otherwise normal lives, until they are exposed to volatile anesthetics; thus this is perhaps the best documented example of an interaction between chemicals and channelopathies. In malignant hyperthermia a missense mutation in the ryanodine receptor and exposure to volatile anesthetics can produce a rapid increase in body temperature which, unless properly treated, can result in death. The underlying cause of this syndrome is thought to be due to excess intracellular Ca^{2+} in myocytes which over time places increased metabolic demand on these cells. Heat is generated due to ATP-depletion and muscle contraction due to this metabolic stress (Betzenhauser and Marks, 2010). This potentially life-threatening syndrome illustrates one way in which gene x environment interaction is important to human health. He also noted that a number of different pesticides are known to interact directly with ion channels, and demonstrated using pyrethroids that the interaction of a pesticide with an ion channel, and the toxicological outcome, can be dramatically influenced by the sequence of peptides in the channel. However, whether or not individuals (or animal models) with sodium channelopathies are more sensitive to pyrethroid neurotoxicity has not been explored to date. The same is true for many other ion channelopathies. Thus, the potential for individuals with channelopathies to be more sensitive to effects of environmental chemicals needs to be investigated further. Dr. Shafer pointed out that the question can be assessed at a number of different levels, as interactions can be readily assessed at the level of the ion channel (native and channelopathy form) and in animal models. Clinical investigations could include epidemiological studies investigating, more specifically, the sensitivity of individuals with channelopathies, or could include generation of inducible stem cells from patient populations for *in vitro* assessment of their sensitivity to chemicals compared to those from control populations.

Dr. William Atchison's presentation focused on calcium channels, their role in disease and toxicity as well as calcium channelopathies. As mentioned previously calcium plays many roles in the cell, such as intracellular signaling and release of neurotransmitters. Because of the many roles calcium plays in the cell its regulation is tightly controlled and VGCCs represent one control mechanism. VGCC are divided into three groups (Ca_v1 – Ca_v3) which result from separate gene products and differ in functional properties, chemical sensitivity, and cellular localization (Dolphin, 2009). Disturbances in VGCCs and therefore calcium dynamics can result in profound disruption of normal cellular function. Environmental neurotoxicants Pb^{2+} and Hg^{2+} disrupt VGCC function and also enter the cell to further disrupt neuronal function, through VCGG. Like the other voltage-gated ion channels, mutations of VGCC that produce pathological changes in neuronal function have been documented in animals and humans. Mutations in CACNA1A, which encodes for the $\alpha1A$ subunit of P/Q-type Ca^{2+} channels, lead to symptoms seen in familial hemiplegic migraine, episodic ataxia type 2, and spinocerebellar ataxia type 6. Conversely, autoimmune attack on Ca^{2+} channels at motor axon terminals causes peripheral cholinergic nerve dysfunction observed in Lambert-Eaton Myasthenic Syndrome (Striessnig et al., 2010). There are established cases of gene X environment interactions in which the symptoms of the channelopathy only occur in response to an environmental trigger. A missense mutation in CACNA1S in skeletal muscle can produce hypokalemic periodic paralysis in response to low serum potassium (Kullmann, 2010). As stated previously gene x environment

interaction also plays a role in malignant hyperthermia. Dr. Isaac Pessah presented his work examining the effects of non-coplanar polychlorinated biphenyls (PCBs) and the ryanodine receptor (RyR). PCBs were once a widely used class of compounds, are persistent in the environment and are a human health concern due to reports of developmental neurotoxicity associated with environmental exposure to PCBs (Tilson and Kodavanti, 1998).

Of particular importance are chronic low-level PCB exposures, especially in populations that live near legacy sources of PCBs and sites of PCB disposal. Epidemiological studies indicate that in addition to developmental neurotoxicity, PCB levels are positively associated with immune system dysfunction and cardiovascular disease. Previous studies have focused on the effects of PCBs on the AhR and assigned a factor to compare the effects to TCDD, the stereotypical AhR agonist. However this particular model for PCB toxicity does not take into account the non-AhR based mechanisms which also have been widely studied and proposed to be important in PCB neurotoxicity (Schantz, 1996, Seegal, 1997, Tilson and Kodavanti, 1998). Dr. Pessah's work has focused on ryanodine receptors (RyR) and alterations in their function by genetic mutation. His work on these RYR mutations has led to studying the gene X environment interactions possible with these channelopathies, as RYR can be activated by non-coplanar PCBs.(Pessah et al, 2010)

The RyR are a family of intracellular Ca^{2+} channels that regulate the release of Ca^{2+} from intracellular stores. RyRs are expressed in most cell types where they modulate intracellular Ca^{2+} signaling to regulate cellular growth, movement, metabolism, secretion and plasticity. Homologous mutations in RyR1 and RyR2 produce unique syndromes in humans; malignant hyperthermia and catecholaminergic polymorphic ventricular tachycardia. These syndromes can result in lethality, and unlike many other channelopathies, may not become expressed until exposure to an environmental trigger such as halogenated anesthetics in malignant hypothermia and temperature or exercise stress in catecholaminergic polymorphic ventricular tachycardia put an individual's life in danger (Pessah et al., 2010).

The work presented by Dr. Pessah addressed the interactions of PCBs and wild-type RyR as well as in mutated RyR and showed the increased activation of mutated RyR in response to PCB exposure. It also serves to explain how non-coplanar PCBs, which do not interact with the AhR, can produce profound biological effects. Using knock-in mice susceptible to malignant hyperthermia (R163C-RyR1) and catecholaminergic polymorphic ventricular tachycardia (R176Q-RyR2) it was shown that in muscle cells isolated from these animals were more than 10 times more sensitive to RyR activation by the non-coplanar PCB 95 than wild-type controls. Embryonic myotubes containing these mutations also showed excessive Ca^{2+} signaling in response to electrical stimuli as well as intracellular Ca^{2+} depletion, which was not seen in WT or in the absence of PCB 95 (Pessah et al., 2010). This research illustrates one way in which gene X environment interactions can produce increased responses to toxic agents *in vitro*. This research suggests that there could be translational applications, as there are a large number of known RyR mutations in humans and PCBs continue to be a human exposure risk. Linking *in vitro* data generated in myocytes to human neurotoxicology is problematic so more research on this highly relevant topic is needed.

Dr. April Neal presented her work on the differential effects of allethrin on VGCC subtypes in rat PC12 cells. Pyrethroid insecticides are one of the most widely used classes of insecticides in the world. Pyrethroids act on target and non-target species by prolonging the open state of the voltage-gated sodium channel (VGSC) and delaying channel inactivation, resulting in a prolonged depolarizing tail current that can lead to increased excitability. The pyrethroids are divided into two classes based their clinical signs at high doses, and these correspond well with the presence of a cyano group: type II compounds have this cyano group and produce choreoathetosis and excessive salivation, whereas type I agents do not

and produce tremor. Differences in the toxicity of the two classes of compounds prompted studies to investigate whether pyrethroids have targets other than the VGSC (Soderlund et al., 2002); among the proposed additional targets are VGCC. Data regarding effects of pyrethroids on VGCC are inconsistent (Shafer and Meyer, 2004), and only a limited number of studies have been performed where pyrethroid effects on VGCC were examined using patch clamp techniques in mammalian neurons.

Using electrophysiological techniques this research showed that allethrin, a type I pyrethroid, increased whole-cell VGCC currents during depolarization relative to control. These effects appeared to be concentration-dependent and were VGSC-independent as 0.5 μ M tetrodotoxin (TTX), a VGSC antagonist, had no effect on the Ca²⁺ current. In order to eliminate the possibility that TTX insensitive VGSC could be driving the effect on Ca²⁺ current, 100 μ M Cd²⁺, a nonspecific VGCC blocker, was applied and completely abolished the effects of allethrin on the whole cell calcium current.

Calcium channels are known to serve a number of functions in the cell, with different isoforms having very specific biological effects. In order to better understand if allethrin was modifying a certain type of channel, thereby having the potential to alter a unique set of biological processes, Dr. Neal pharmacologically isolated isoform specific Ca²⁺ currents. The greatest effect was seen with GVIA, an N-type VGCC antagonist, suggesting that the allethrin-induced Ca²⁺ currents are mainly produced by N-type calcium channels (Neal et al., 2010).

Dr. Neal's research suggests that allethrin differentially affects VGCC subtypes, which may interfere with normal calcium dynamics in cells. This could have profound effects on neuronal development and function and warrants further research into the effects of pyrethroids on VGCCs. This research also suggests that individuals with calcium channel mutations could potentially be more susceptible to the toxic effects of pyrethroids that act on VGCCs.

Mr. Jason Magby presented his research examining the effects of developmental deltamethrin exposure on expression of VGSC mRNA *in vivo* and *in vitro*. Previous *in vitro* studies have demonstrated that exposure to VGSC agonists such as scorpion toxin and veratridine results in down-regulation of VGSC protein (Dargent and Couraud, 1990) and mRNA (Lara et al., 1996). However this has never been demonstrated *in vivo*. The pyrethroids provide a unique set of compounds because they are known VGSC agonists, are not as toxic as traditional VGSC agonists and are commonly found in household and industrial use. The pyrethroid pesticide deltamethrin was given to pregnant C57B6/J mice throughout gestation and lactation (3mg/kg, every 3 days) and the brains of the offspring were harvested 10–11 months later for analysis by qRT-PCR. VGSC mRNA was shown to be reduced in the frontal cortex and striatum of both male and female mice exposed to deltamethrin. In an *in vitro* system used to determine the mechanism of this down-regulation, this same down-regulation of mRNA could be reproduced with 24 hour exposure to 100 nM deltamethrin and could be blocked by pre-exposure to the voltage-gated sodium channel antagonist tetrodotoxin (TTX) or the intracellular Ca²⁺ chelator BAPTA-AM. These results indicate a role for interaction of deltamethrin with the VGSC, as well as intracellular Ca²⁺ signaling. Because studies have recently shown that calpain is involved in the regulation of VGSC protein, the role of calpain activation on the down-regulation of VGSC mRNA was tested. Pre-exposure to the calpain inhibitor PD 150606 prevented the down-regulation of VGSC mRNA suggesting a role for calpain activation in the deltamethrin induced down-regulation of VGSC mRNA. This research suggests that developmental exposure to VGSC agonists can result in persistent mRNA down-regulation, which could have profound effects on neuronal depolarization. It is possible that this alteration in VGSC

expression and subsequent alterations in neuronal excitability could produce pathologies similar to what is seen in channelopathies arising from genetic mutations. Additionally, individuals with an ion channel mutation that does not manifest clinically could be exacerbated as a result of exposure to agents that act on ion channels and alter their expression.

Ion channels provide critical control of the function of electrically excitable tissues. As such, they are targets of a wide variety of natural toxins, pharmaceutical compounds, and toxic chemicals. In the past decade, significant new discoveries have demonstrated that mutations in ion channel sequences are associated with specific, clinically-definable syndromes. While there are some specific examples of drug x gene interactions that can result in dramatic clinical outcomes, there have not been any significant studies of whether or not these channelopathies may also interact with environmental chemicals in a similar fashion. This session identified a number of specific research questions related to chemical interactions with channelopathies that need to be addressed, including:

- Are individuals with channelopathies more sensitive to environmental compounds that act on ion channels?
- Which ion channel mutations are the most relevant in a human population when with respect to environmental exposures?
- Could environmental exposures trigger, or unmask symptoms of channelopathies that have been dormant, or exacerbate symptoms once they have been manifested?
- What is the role of channelopathies in neurodegenerative diseases and do environmental exposures exacerbate these diseases?
- Do individuals with channelopathies differ in their responses to the persistent and/or developmental effects of agents that act on ion channels?

As pointed out by Dr. Shafer in the introductory presentation, ion channels with the mutations associated with channelopathies have been cloned and are available for use in *in vitro* studies of chemical interactions with mutant channels. In addition, several mouse models are also available that are good animal models of these channelopathies. Clinical populations may also allow for some limited opportunities to study interactions between environmental agents and channelopathies. Thus, the question of whether there are gene x environment interactions in the case of individuals with channelopathies can be readily addressed. Although these conditions are not as prevalent as other diseases, a better understanding of the potential interactions of environmental chemicals with channelopathies could have profound ramifications for affected individuals because of the acute nature and severity of effects with channelopathies.

References

- Betzenhauser MJ, Marks AR. Ryanodine receptor channelopathies. *Pflugers Arch.* 2010; 460:467–480. [PubMed: 20179962]
- Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron.* 2000; 26:13–25. [PubMed: 10798388]
- Dargent B, Couraud F. Down-regulation of voltage-dependent sodium channels initiated by sodium influx in developing neurons. *Proc Natl Acad Sci U S A.* 1990; 87:5907–5911. [PubMed: 2165609]
- Dolphin AC. Calcium channel diversity: multiple roles of calcium channel subunits. *Curr Opin Neurobiol.* 2009; 19(3):237–44. [PubMed: 19559597]
- Kullmann DM. Neurological channelopathies. *Annu Rev Neurosci.* 2010; 33:151–172. [PubMed: 20331364]

- Lara A, Dargent B, Julien F, Alcaraz G, Tricaud N, Couraud F, Jover E. Channel activators reduce the expression of sodium channel alpha-subunit mRNA in developing neurons. *Brain Res Mol Brain Res.* 1996; 37:116–124. [PubMed: 8738142]
- Modell SM, Lehmann MH. The long QT syndrome family of cardiac ion channelopathies: a HuGE review. *Genet Med.* 2006; 8:143–155. [PubMed: 16540748]
- Neal AP, Yuan Y, Atchison WD. Allethrin differentially modulates voltage-gated calcium channel subtypes in rat PC12 cells. *Toxicol Sci.* 2010; 116:604–613. [PubMed: 20466778]
- Pessah IN, Cherednichenko G, Lein PJ. Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. *Pharmacol Ther.* 2010; 125:260–285. [PubMed: 19931307]
- Schantz SL. Developmental neurotoxicity of PCBs in humans: what do we know and where do we go from here? *Neurotoxicology and Teratology.* 1996; 18(3):217–27. [PubMed: 8725628]
- Seegal RF. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit Rev Toxicol.* 1997; 26(6):709–37. [PubMed: 8958469]
- Striessnig J, Bolz HJ, Koschak A. Channelopathies in Cav1.1, Cav1.3, and Cav1.4 voltage-gated L-type Ca²⁺ channels. *Pflugers Arch.* 2010; 460:361–374. [PubMed: 20213496]
- Tilson HA, Kodavanti PR. 1998; The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology.* 19(4–5):517–25. [PubMed: 9745906]
- Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol Rev.* 2008; 88:1407–1447. [PubMed: 18923186]
- Waxman SG. Channel, neuronal and clinical function in sodium channelopathies: from genotype to phenotype. *Nat Neurosci.* 2007; 10:405–409. [PubMed: 17387329]

Table 1

Ion Channels and Selected Agents That Act on Them

Channel	Gene	Isoform	Diseases ^a	Environmental/Pharmacological Agents ^b
Sodium channels	SCN1A	Na _v 1.1 (α subunit)	Epilepsy, migraine	Pyrethroid pesticides, brevetoxins, organochlorine pesticides, volatile organic compounds (e.g. toluene) local anesthetics (ex. procaine, bupivacaine), anti-convulsants (ex. Phenytoin), antiarrhythmic agents (ex. Procainamide)
Potassium channels	SCN1B	β1	Epilepsy	
	SCN2A	Nav1.2 (α subunit)	Epilepsy	
	KCNQ2	Kv7.2	Epilepsy	apitoxin , antiarrhythmic agents (ex. amiodarone)
	KCNQ3	Kv7.3	Epilepsy	
	KCNMA1	BK	Epilepsy with dyskinesia	
	KCNA1	Kv1.1	Episodic ataxia	
	KCNC3	Kv3.3	Ataxia	
Calcium channels	CACNA1H	α1H subunit of Cav3.2	Epilepsy	Mercury (including methylmercury), lead, cadmium, nickel cobalt, pyrethroids antihypertensives (ex. dihydropyridines), antiarrhythmic agents (ex. Verapamil)
	CACNA1A	α1A subunit of Cav2.1	Episodic or progressive ataxia, migraine, epilepsy	
GABAA receptors	GABRA1	α1	Epilepsy	Phenylpyrazoles, proconvulsant drugs, sedatives (benzodiazepines, ethanol), cyclodiene insecticides (e.g. lindane), RDX, volatile organic compounds (e.g. toluene), metals including mercury, antianxiolytics, muscle relaxants and volatile anesthetics
	GABRB3	β3	Epilepsy	
Nicotinic ACh receptors	GABRG2	γ 2	Epilepsy	
	CHRNA2	α2	Epilepsy	Nicotine, neonicotinoid pesticides, volatile organic compounds (e.g. toluene) paralytic drugs, antidepressant, antismoking drugs
	CHNRA4	α4	Epilepsy	
Glycine receptors RYR receptor	CHRNB2	β2	Epilepsy	
	GLRA1	α1	Hyperekplexia	Phencyclidine, volatile organic compounds (e.g. toluene) and ketamine
	RYR1		malignant hyperthermia, congenital myopathy	PCBs, caffeine, volatile anesthetics
	RYR2		arrhythmogenic disorders	

^aThis column lists examples of diseases or clinical syndromes that have been linked to mutations in this ion channel subunit.

^bThis column lists examples of environmental and/or pharmacological agents that are known to interact with these channels. These lists of examples may not include all compounds known to act at a given channel type.