CURRENT TOPIC

Ion channels and neurology

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Rapid communication between cells is dependent on electrical signals produced by the passage of charged ions through specialised proteins embedded in cell membranes.¹ These ion channels have been essential to the health and survival of organisms since life began. One might therefore expect that diseases of ion channels or channelopathies would not be compatible with life. The past decade has shown this not to be the case with a rapidly expanding list of disorders, many of which have their onset in childhood and affect the nervous system and muscle. Abnormalities of ion channel function are responsible for a variety of non-neurological disorders including Bartter syndrome, X linked nephrolithiasis, neonatal hyperinsulinism, long QT syndromes, and most notably defects in function of the CFTR chloride channel gene causing cystic fibrosis.

Many of the neurological channelopathies recognised to date are rare forms of common diseases and potentially give us clues to disease mechanisms in common disorders such as epilepsy and migraine. Some of the disorders are common but unrecognised or, because of their paroxysmal nature, misdiagnosed.

One of the most exciting aspects of work in this field is that collaboration between clinicians, molecular geneticists, and cell physiologists is providing new insights into the biological basis of diseases. The fruits of these collaborations include recognition that diseases caused by ion channel dysfunction may have genetic and autoimmune aetiologies.

Basic science of ion channel function

Ion channels are made up of subunits, which combine to form structures with a central aqueous pore. Channels are selective for particular ion species and can be gated, altering conformation to open or close. The two principal classes are voltage and ligand gated channels.

The action potential, which is central to normal function within the nervous system, is entirely dependent on voltage gated ion channels. As the transmembrane voltage changes a voltage gated sodium channel opens and ions pass rapidly down an electrochemical gradient from a high concentration outside the cell through the channel pore into the cell. The normally negatively charged cell interior depolarises, causing voltage gated potassium channels to open and potassium ions to leave the cell, restoring the resting membrane potential. This sequential depolarisation and repolarisation of the cell membrane is the fundamental basis of the action potential.²

A ligand gated ion channel opens following binding of a ligand to the receptor portion of the channel. Other factors influencing ion channel function include intracellular messengers, phosphorylation of channel residues, pH, and temperature. This multitude of potentially influential variables may explain the paroxysmal nature of many of the genetic channelopathies and their propensity to be precipitated by a variety of factors.

In an effort to understand the functional consequences of particular channel mutations, cDNA techniques have been utilised to express mutant and normal channels in *in vitro* biological systems, allowing currents to be measured across either groups of channels in membrane patches or single channels.

Central nervous system channelopathies

Table 1 presents a classification of CNS channelopathies.

EPILEPSY

At least 60% of childhood epilepsies are termed idiopathic—that is, not related to a structural brain lesion and with a strong genetic influence to their expression. By studying large families and adopting a syndromic approach to epilepsy classification, several genes causing idiopathic epilepsies have been identified. All these genes code for ion channels.

Table 1 Central nervous system channelopathies

Ion channel	Disorder	Inheritance	Gene	Chromosome
<i>Voltage gated</i>				
Sodium, β 1 subunit	GEFS+	Dominant	SCN1B	19q
Sodium, α 1 subunit	GEFS+	Dominant	SCN1A	2q
Potassium	BNFC	Dominant	KCNQ2	20q
Potassium	BNFC	Dominant	KCNQ3	8q
Potassium	EA1, EA1 and epilepsy, epilepsy and myokymia	Dominant	Kv1.1	12p
Calcium	FHM, SCA6, EA2	Dominant	CACNL1A4	19p
<i>Ligand gated</i>				
Neuronal nicotinic acetylcholine receptor, α 4 subunit	ADNFLE	Dominant	CHRNA4	20q
Neuronal nicotinic acetylcholine receptor, β 2 subunit	ADNFLE	Dominant	CHRNA2	1p
Glycine receptor	Hyperekplexia	Dominant, recessive	GLRA-1	5q

GEFS+, generalised epilepsy with febrile seizures plus; BNFC, benign neonatal familial convulsions; EA1, episodic ataxia type 1; FHM, familial hemiplegic migraine; SCA6, spinocerebellar ataxia type 6; EA2, episodic ataxia type 2; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy.

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is not a rare syndrome. Symptoms may be mild, with only a few seizures in childhood, or severely disabling with 50 seizures in a single night, refractory to anti-epileptic medication. Presentation is in childhood with brief partial seizures arising from sleep. These are characterised by arousal, dystonic posturing of one or more limbs, clonic jerking, screaming, and kicking of the legs.³ Seizures may be misdiagnosed as night terrors or psychological problems. Interictal and ictal surface EEG is usually normal as the discharges arise in deep cortical regions. The most helpful investigation is home video of the events and the most effective medication for the syndrome is carbamazepine.

A detailed study of a Melbourne family led to the discovery of the first genetic cause of an idiopathic epilepsy, a point mutation in the $\alpha 4$ subunit of the neuronal nicotinic acetylcholine receptor.⁴ Most ADNFLE families do not link to this locus, suggesting that although the clinical phenotype is fairly uniform, there is a heterogeneous genetic aetiology. Expression studies of mutant channels have shown a loss in function.⁵ It can be hypothesised, as these receptors are principally located presynaptically, that channel dysfunction leads to abnormalities of inhibitory neuronal networks in sleep and thus to the epilepsy.

Benign familial neonatal convulsions (BFNC) is a dominant epilepsy syndrome presenting in the first week of life with brief seizures involving tonic extensions, head and eye deviation, apnoea, and clonic limb movements in otherwise well infants. The epilepsy remits within days, weeks, or months, although 10% of cases will have seizures when older. Ictal EEG shows onset in one or other hemispheres. This, like ADNFLE, is a non-lesional partial epilepsy syndrome, not a generalised epilepsy as previously thought.

Mutations in one of two voltage gated potassium channel subunit genes, KCNQ2 and KCNQ3, cause BNFC.^{6,7} A slow, repolarising brain current, the "M" current, flows through a channel made up by the KCNQ2 and KCNQ3 subunits coassembling. Functional studies on mutant subunits show a partial reduction in the M current.⁸ A reduction in critical current amplitude through this channel at a physiologically vulnerable age leaves neurones relatively depolarised and excitable, producing a benign, neonatal epilepsy.

Mutations in other members of the KCNQ family of channels are responsible for a form of the long QT syndrome and inherited deafness. An "arrhythmia" of the brain shares a similar aetiology to an "epilepsy" of the heart. The endolymph of the cochlear requires a very specific potassium concentration and therefore mutations in channels, which maintain this concentration, may cause deafness.

Generalised epilepsy with febrile seizures plus (GEFS+) is a common, dominant, epilepsy syndrome with variable penetrance in which family members express a variety of generalised epilepsy phenotypes.^{9,10} These include typical febrile seizures, febrile seizures beyond six

years, febrile seizures and afebrile generalised tonic-clonic seizures, febrile seizures and absences, myoclonic astatic epilepsy, and severe myoclonic epilepsy of infancy. The familial nature of the epilepsy may remain unrecognised unless there is a family member with a more severe epilepsy phenotype. The commonest phenotype is simple febrile seizures. A Tasmanian family showed linkage to chromosome 19q and a mutation was discovered in the voltage gated sodium channel $\beta 1$ subunit gene.¹¹ Expression studies show that this mutation results in a loss of function of the sodium channel. Recently mutations in the $\alpha 1A$ voltage gated sodium channel subunit have been shown to segregate with disease in GEFS+.¹²

MIGRAINE

Familial hemiplegic migraine (FHM) is a subtype of dominantly inherited migraine with aura in which attacks of hemiplegia, typically lasting 30–60 minutes, begin in the aura phase. In several pedigrees with FHM, missense mutations in a voltage gated calcium channel gene, CACLN1A4, segregate with the disease.¹³ In FHM families there are individuals who have classical migraine with aura, without hemiplegia, who have the calcium channel mutation.

MOVEMENT DISORDERS

Mutations in the CACNL1A4 gene are responsible for two other dominantly inherited disorders, *spinocerebellar ataxia type 6* (SCA6) and *episodic ataxia type 2* (EA2).¹⁴ SCA6 is an adult onset, progressive, cerebellar ataxia with cerebellar degeneration associated with a CAG triplet repeat insertion in the gene, resulting in an expanded polyglutamine tract in the C terminal region of the channel protein. Chronic disruption of calcium homeostasis may be the cause of the cerebellar degeneration in SCA6 patients. EA2 is a paroxysmal ataxia with onset in childhood, characterised by episodes of cerebellar ataxia and nystagmus lasting hours or days, precipitated by stress or exercise. Between episodes individuals may have cerebellar signs and in some cases a slowly progressive ataxia. Conversely individuals with SCA6 may present with a paroxysmal ataxia. Headache may be a feature in all three disorders associated with CACNL1A4 mutations, illustrating that though they are clinically distinct in their "pure" forms, overlap exists and the parallels with common forms of migraine are notable.

A voltage gated potassium channel, Kv1.1, is expressed in cerebral cortex, cerebellum, and peripheral nerve. Channel dysfunction is reflected in symptoms related to each region. Mutations in this gene cause *episodic ataxia type 1* (EA1).¹⁵ Affected individuals have cerebellar ataxia lasting seconds or minutes, precipitated by sudden movement or emotion. Hyperexcitability of peripheral nerves causes subtle, rippling muscle movements (myokymia) and in more severe cases neuromyotonia and flexion contractures of limbs, so infants may be misdiagnosed as having cerebral palsy. Some individuals have epilepsy reflecting cortical

Table 2 Skeletal muscle and nerve channelopathies

Ion channel	Disorder	Inheritance	Gene	Chromosome
Voltage gated Potassium	Familial generalised myokymia	Dominant	Kv1.1	12p
Sodium	HyperPP	Dominant	SCNA4	17q
	Paramyotonia congenita	Dominant		
Calcium	PAM	Dominant		
	HypoPP	Dominant	CACNL1A3	1q
Calcium	Malignant hyperthermia	Dominant		
	Central core disease	Dominant	RYR1	19q
	Malignant hyperthermia	Dominant		
Chloride	Myotonia congenita		CLCN1	7q
	Thomsen's disease	Dominant		
	Becker's myotonia	Recessive		
Potassium (cochlea)	Jervell and Lange-Nielsen syndrome	Recessive	KCNQ1	11p
	(Long QT and deafness)	Recessive	KCNE1	21q
	Autosomal dominant deafness type 2	Dominant	KCNQ4	1p
Calcium (retina)	Stationary night blindness	X linked	CACNA1F	Xp
Ligand gated Nicotinic acetylcholine receptor	Congenital myasthenia	Dominant and recessive	CHRNA CHRN CHRN CHRN	2q 17p

HyperPP, hyperkalaemic periodic paralysis; PAM, potassium aggravated myotonia; HypoPP, hypokalaemic periodic paralysis.

dysfunction.¹⁶ There is considerable variability in the phenotypic expression of mutations in this gene, such that presentation may vary from "idiopathic" toe walking to complex partial epileptic seizures.¹⁷

HYPEREKPLEXIA

Hyperekplexia is characterised by excessive startle responses. Infants have hypertonia when awake and in response to a minor stimulus can have periods of generalised muscle contraction leading to apnoea and life threatening syncope. Older children and adults continue to have excessive startle leading to falls. Glycine is the principal inhibitory neurotransmitter in the spinal cord. Hyperekplexia is caused by dominant and recessive mutations in the glycine receptor, GLRA1, a ligand gated chloride channel.¹⁸

Neuromuscular channelopathies

Table 2 presents a classification of skeletal muscle and nerve channelopathies.

When an action potential arrives at the presynaptic terminal, voltage gated calcium channels are activated, allowing calcium to enter the terminal and trigger the release of acetylcholine into the synaptic cleft. In the *Lambert-Eaton myasthenic syndrome* antibodies are directed against these calcium channels, resulting in reduced acetylcholine release.

Autoimmune myasthenia gravis has long been known to be caused by antibodies directed against subunits of the acetylcholine receptor. *Transient neonatal myasthenia* and *recurrent neonatal arthrogryposis* (secondary to reduced fetal movement in utero) may be caused by transplacental transfer of these maternal antibodies. These paediatric disorders can be prevented or ameliorated by appropriate treatment of the mother.

Congenital myasthenic syndromes are caused by a variety of abnormalities in neuromuscular transmission.¹⁹ In the *slow channel syndrome*, dominantly inherited mutations in channel

subunits lead to prolonged channel opening and desensitisation of the postsynaptic membrane. The onset and severity of symptoms are determined by the site of the mutation. As this disorder is caused by an abnormally prolonged response to acetylcholine it is unresponsive to acetylcholinesterase inhibitors, responding instead to the channel blocker quinidine.

In the *fast channel recessive syndromes* there is a reduced response to acetylcholine with mutations resulting in either reduced affinity to acetylcholine at the receptor binding site, reduced probability of the channel opening, the channel opening and closing too rapidly, or a reduction in total receptor numbers.

Once acetylcholine has bound to the receptor portion of the channel, cations, principally sodium, enter the cell causing membrane depolarisation. This in turn activates voltage-gated sodium channels and the action potential spreads across the muscle fibre causing T tubule calcium channels to activate and in turn trigger the release of calcium from the sarcoplasmic reticulum and muscle contraction. The muscle membrane is then returned to resting potential by the action of voltage gated chloride channels.

Inherited myotonias are caused by sodium and chloride channel mutations.²⁰ *Myotonia congenita* is characterised by attacks of muscle stiffness that are usually painless and are relieved by exercise (the so called warm up effect). It exists in a dominant phenotype (*Thomsen's disease*) and a more severe recessive form (*Becker's myotonia*). Both are caused by mutations in a chloride channel, CLCN1. Mutations lead to a reduction in chloride ions entering the cell so it remains relatively depolarised, resulting in spontaneous oscillations in membrane potential and clinical myotonia. The symptoms may respond to the class I antiarrhythmic drug mexilitene.

Paramyotonia congenita is a dominantly inherited condition in which myotonia paradoxically worsens with exercise and exposure to low temperatures. Mutations in the voltage gated sodium channel, SCNA4, underlie this disorder. Channels open later or for longer after depolarisation, so that persistent sodium flux initiates repeated depolarisations. Different mutations in the SCNA4 gene are responsible for hyperkalaemic periodic paralysis. This may exist as a distinct syndrome from paramyotonia congenita or in combination with it. These channels have impaired fast inactivation with prolonged sodium currents leading to desensitisation of the membrane and inactivity of a proportion of the channels. The failure of repolarisation leads to accumulation of potassium in the extracellular space.

Hypokalaemic periodic paralysis is caused by mutations in the voltage gated calcium channel CACNL1A3, although the precise mechanism is unclear. Mutation in this gene may also be responsible for the potentially fatal *malignant hyperthermia* syndrome; however, a more common form of this syndrome is caused by mutations in the ryanodine voltage gated channel gene.²¹ The ryanodine channel allows calcium to leave the sarcoplasmic reticulum to initiate

muscle contraction. The mutant channel, when exposed to volatile anaesthetics or depolarising muscle relaxants, allows excess calcium out of the sarcoplasmic reticulum resulting in hyperthermia, muscle rigidity and rhabdomyolysis. DNA diagnosis should reduce the need for invasive provocation studies (the in vitro contracture test) on muscle biopsies from potentially affected individuals.

Conclusion

The spectrum of neurological channelopathies continues to expand. In our view the majority of the idiopathic epilepsy syndromes are likely to be channelopathies. A particular clinical phenotype such as an epilepsy syndrome may be the consequence of dysfunction in more than one ion channel in an individual. The role that variation in gene expression plays in the age related phenotypes of these disorders is likely to be better understood in the future.

The critical role of ion channels means that they are conserved throughout evolution, allowing us to gain insights from homologous channels in animal models of human disease.²²

DNA based diagnosis is now possible for many of the ion channel disorders, although the process may be complex as the number of potential mutations within a single gene may be high and the same clinical disorder may be caused by mutations in different genes. In view of their often paroxysmal nature, misdiagnosis and labelling of symptoms as psychological is a common feature of ion channel disorders. A meticulous history remains the cornerstone of the diagnostic process. With the current pace of scientific progress it is inevitable that several new ion channel disorders will be described in the next decade. Many will be clearly paroxysmal but we suspect that channel dysfunction will be increasingly implicated in more chronic neurological and possibly neuropsychiatric disorders.

As the biological basis of each disease is understood it opens up exciting prospects for pharmacological and genetic therapies targeted to specific ion channel defects.

Addendum

Mutations in the beta 2 subunit of the neuronal nicotinic acetylcholine receptor have recently been shown to be associated with ADNFLE in an Italian family²³ and a large Scottish family.²⁴ The epilepsy phenotype seems to be identical to that caused by CHRNB4 mutations.

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