Physiologic Alterations in Ataxia

Channeling Changes Into Novel Therapies

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The ataxias constitute a heterogeneous group of diseases in which cerebellar dysfunction typically underlies the major neurologic manifestations. It is increasingly clear that ataxia can result directly from mutations in ion channels or from perturbations in ion channel physiology in the absence of a primary channel defect. Neuronal dysfunction stemming from perturbed channel activity likely explains some motor deficits in episodic and degenerative ataxias. Understanding these pathophysiologic changes may reveal novel therapeutic targets for symptomatic treatment of ataxia.

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Ion channels form pores in cell membranes through which biologically relevant ions pass selectively. Channels play a central role in regulating neuronal electrical excitability, and their dysfunction underlies many neurological disorders including various forms of periodic paralyses, myotonic disorders, and epilepsies. Perturbations in channels are increasingly recognized to underlie ataxic disorders as well. Here we review the known alterations in physiology that underlie ataxic disorders.

Two major categories of ion channels exist: voltage-gated and ligand-gated. Voltage-gated channels on the cell surface generate ionic currents in response to changes in membrane potential. Ligand-gated channels are opened instead by neurotransmitters and are often localized to synapses. Synaptic channels include glutamate, \( \gamma \)-aminobutyric acid (GABA), acetylcholine, and glycine receptors. Additional ligand-gated channels are found intracellularly including the inositol 1,4,5-trisphosphate (InsP\(_3\)) receptor and ryanodine receptor in the endoplasmic reticulum. Channelopathies resulting directly from mutations in ion channels underlie some forms of cerebellar ataxia. In addition, alterations in ion channel localization or availability in the absence of any channel mutation can profoundly alter cerebellar neuronal excitability, resulting in ataxia.

Both ataxia-causing channelopathies and inherited ataxic disorders associated with ion channel dysfunction can result from gene mutations inherited in an autosomal dominant pattern. These mutations can cause disease symptoms through 1 of 3 mechanisms: haploinsufficiency, dominant-negative effects, or toxic gain-of-function effects. In the case of haploinsufficiency, the mutated protein is non-functional or defective, and insufficient levels of functional protein lead to neuronal dysfunction. Dominant-negative effects arise from mutations in proteins that participate in multimeric protein complexes; assembly of mutant protein into these complexes disrupts the function of the entire complex. Dominant toxic mutations confer a deleterious new function on the mutated protein. In many cases this new toxic function is associated with protein misfolding. All 3 types of mutations have been described in the inherited ataxic syndromes.

Ataxic disorders comprise a wide and heterogeneous group of diseases, with cerebellar dysfunction typically being the major neurologic feature. The cerebellum receives excitatory input through mossy and climbing fibers. This input is mostly channeled into Purkinje neurons, the only projection neurons from the cerebellar cor-

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Although cerebellar neuronal loss is a common feature in degenerative ataxias, it is not necessary for motor incoordination. Indeed, in certain mouse models of ataxia and in some human episodic ataxias, physiologic alterations in cerebellar neurons can cause a profound motor dysfunction as neuronal loss.1,2

As important regulators of neuronal excitability, ion channels are ideally poised to modulate cerebellar physiology. Several ion channels help regulate Purkinje neuron firing. The sodium channel Nav1.6 is the major depolarizing conductance required to sustain intrinsic firing in Purkinje neurons. Further depolarization resulting from activation of glutamate receptors can increase Purkinje cell intrinsic firing frequency while activation of GABA\(_A\) receptors reduces this firing frequency. Ion channel dysfunction in the cerebellum would thus be expected to alter cerebellar circuit properties, resulting in motor dysfunction (Table 1 and Table 2; Figure 1). For example, an impaired capacity of Purkinje neurons either to sustain repetitive firing or to modulate intrinsic firing in response to synaptic input has been observed in mouse models of spinocerebellar ataxia type 13 (SCA13), episodic ataxia 2 (EA2), severe myoclonic epilepsy of infancy (SMEI), and SCA27. Alterations in synaptic transmission at the parallel fiber–Purkinje neuron synapse may contribute to the ataxia in SCA15, SCA5, dentatorubral pallidolysian atrophy (DRPLA), and the ataxia associated with the paraneoplastic Lambert-Eaton myasthenic syndrome. Finally, alterations in basket cell physiology appear to be responsible for EA1.

### ATAXIC CHANNELOPATHIES

#### Spino cerebellar Ataxia Type 6

One of the 9 known CAG repeat/polyglutamine disorders, SCA6 is caused by the expansion of a polyglutamine tract in the carboxyl terminus (C-terminus) of the P/Q-type voltage-gated calcium channel alpha subunit, Cav2.1. A relatively “pure” cerebellar syndrome, SCA6 is notable for selective degeneration of cerebellar Purkinje neurons.
Though the precise mechanism by which the altered Cav2.1 protein causes ataxia remains uncertain, recent evidence suggests both altered channel function (haploinsufficiency) and production of an aberrant C-terminal fragment (gain of function) in SCA6. Depending on the cell system used, transfected cells expressing the mutant (expanded) channel exhibit either increased or decreased calcium entry into the cell. Cerbellar granule cells expressing the mutant channel α subunit showed nuclear localization of a C-terminal fragment containing the polyglutamine stretch. It was surmised that this cleavage fragment caused toxicity in a manner similar to other polyglutamine diseases. In a recently developed mouse model of SCA6, however, no alteration in channel function or truncated C-terminal fragment was noted. Nevertheless, age-related calcium channel dysfunction cannot yet be ruled out, as the electrophysiologic recordings of calcium currents were performed in young mice many months prior to the onset of mild motor dysfunction.

**Spinocerebellar Ataxia Type 13**

A rare form of dominantly inherited ataxia, SCA13 is caused by mutations in the KCNC3 gene encoding the voltage-gated potassium channel protein Kv3.3. Mutations in KCNC3 are presumed to suppress Kv3.3 currents in dominant-negative manner. Patients exhibit truncal and appendicular ataxia, dysarthria, hyperreflexia, and nystagmus. Although Kv3.3 is widely expressed in the brain, the highest levels of expression are in Purkinje neurons.

KCNC3 null mice provide insight into the pathogenic mechanism in SCA13. They show impaired motor performance when traversing a narrow beam and altered firing properties of Purkinje neurons. Purkinje neurons in null mice display broader action potentials, fail to sustain high-frequency firing in response to injected current, and have reduced burst frequency in response to climbing fiber stimulation. The cerebellar atrophy seen in patients with SCA13 has been hypothesized to be due to increased calcium entry into Purkinje neurons secondary to the spike broadening seen in the null mice. Restoring Kv3.3 channels exclusively in Purkinje cells in otherwise KCNC3 null mice rescued the abnormalities in Purkinje neuron firing and motor coordination.

**Spinocerebellar Ataxia Type 15**

Spinocerebellar ataxia type 15 is a progressive neurodegenerative disorder characterized by pure cerebellar ataxia, slow progression, and cerebellar atrophy. It results from mutations in the ITPR1 gene encoding the InsP3 receptor 1. Expressed predominantly in Purkinje neurons, this InsP3 receptor isoform acts as an InsP3-gated Ca2+ release channel. Homozygous and heterozygous ITPR1 null mice develop ataxia in the absence of neurodegeneration. Faster decay of the parallel fiber excitatory postsynaptic current was noted in mutant mice, with no change in intrinsic firing of Purkinje neurons. Decreased modulation of Purkinje neuron intrinsic firing by the excitatory synaptic input from parallel fibers would likely result in decreased inhibition of DCN neurons. Increased DCN excitability has been demonstrated to result in profound ataxia in other mouse models.1

**Episodic Ataxia 1**

Although the episodic ataxias are dominantly inherited like the SCAs, they do not show the progressive neurological cell loss common to the SCAs. The brief attacks of generalized ataxia induced by physical and emotional stress seen in EA1 result from mutations in the voltage-gated potassium channel, KCNA1 (Kv1.1). Mild or subclinical myokymia may be noted in distal musculature. At least 6 mutations in the Kv1.1 channel have been identified in EA1. All of these mutations impair channel function via dominant-negative effects. In a mouse model of EA1, Purkinje neurons showed increased frequency and amplitude of spontaneous GABAergic inhibitory postsynaptic currents. Kv1.1 normally appears to hyperpolarize axon branch points of cerebellar inhibitory basket neurons, thereby preventing some action potentials from reaching the presynaptic terminal of these neurons. A lack of sufficient functional Kv3.3 channels may cause more action potentials to invade the basket neuron presynaptic terminal and hence raise Purkinje neuron inhibitory postsynaptic current frequency. Acetazolamide, a drug used to prevent ataxic episodes in EA1, may reduce excitability of GABAergic interneurons through intracellular alkalinization.

**Episodic Ataxias 2 and 5**

Episodic ataxia 2 is caused by mutations in CACNA1A. It is allelic with SCA6 and familial hemiplegic migraine, 2 other disorders caused by mutations in this gene. More than 20 CACNA1A mutations have been identified in EA2, most of which result in a truncated, nonfunctional protein. Episodic ataxia 2 is characterized by attacks of ataxia and migraine-like symptoms that may be precipitated by physical and emotional stress, coffee, or alcohol. Signs of cerebellar dysfunction can be present between the paroxysmal episodes. Supporting a key role for channel dysfunction in episodic ataxias is the fact that another form of episodic ataxia, EA5, is caused by mutations in CACNB4, which encodes an auxiliary β subunit of Cav2.1.

In EA2, mutations in the α subunit of the P/Q-type voltage-gated calcium channel, Cav2.1, lead to reduced P/Q-type calcium current density. Calcium entry through these channels into presynaptic terminals is required for neurotransmitter release, and impaired synaptic transmission in the cerebellum has been postulated to underlie the motor symptoms of EA2. In the ataxic “tottering” mouse, which has a mutation in the orthologous mouse gene, impaired synaptic transmission at the parallel fiber-Purkinje neuron synapse and reduced precision of intrinsic pacemaker firing by Purkinje neurons have been detected. Importantly, pharmacological correction of this pacemaker impairment with a calcium-activated potassium channel opener improves motor behavior in tottering mice, strongly supporting the view that this electrophysiological abnormality contributes to ataxia. Acetazolamide prevents or attenuates attacks in 50% to 75% of patients. Acetazolamide-
induced changes in intracellular pH and the resulting change in potassium channel conductance may explain the therapeutic effect of the drug.

**Severe Myoclonic Epilepsy in Infancy**

An autosomal dominant disorder, SMEI results from mutations in the SCN1A gene encoding the Nav1.1 channel. The epilepsy in SMEI is accompanied by cognitive deterioration, interictal myoclonus, long tract signs, and ataxia, the latter of which contributes substantially to functional impairment.12

Homozygous Nav1.1 knockout mice develop ataxia and seizures by postnatal day 9 and die by postnatal day 15. Heterozygous knockout animals develop gait abnormalities at postnatal day 21, a developmental time point in mice corresponding to 1 to 2 years of age in children with SMEI, the age when ataxia first appears. Nav1.1 knockout mice exhibit a reduced rate of spontaneous firing in Purkinje neurons. Mutant Purkinje neurons also require stronger depolarizing currents to sustain the same rate of firing.13 Although the major sodium channel isoform responsible for sustaining repetitive firing in Purkinje neurons is Nav1.6, some Nav1.1 is expressed in Purkinje cells and appears to be physiologically important to sustain higher rates of firing than are possible with Nav1.6 alone. Nav.1.1 is widely expressed in other neurons, including hippocampal neurons, which may explain the seizures seen in this disorder.

**ATAXIAS ASSOCIATED WITH SECONDARY ION CHANNEL DYSFUNCTION**

**Spinocerebellar Ataxia Type 27**

Spinocerebellar ataxia type 27 was first described in a Dutch pedigree manifesting childhood-onset postural tremor and slowly progressive ataxia beginning in young adulthood. Orofacial dyskinesias, postural limb tremor, tremor and slowly progressive ataxia beginning in young adults. Spinocerebellar ataxia type 27 was first described in a Dutch pedigree manifesting childhood-onset postural tremor, the ataxic phenotype in this disorder evidently results from mutations in the SCA27 gene. Heterozygous knockouts develop ataxia that resembles human SCA27. Most Purkinje neurons in SCA27 null mice do not fire spontaneously, and both Purkinje and granule cells fail to fire repetitively in response to depolarizing current injections. In Purkinje neurons, this altered firing has been attributed to reduced expression of Nav1.6 protein, the sodium channel necessary for repetitive firing.15 The FGF14 protein normally interacts with voltage-gated sodium channels and appears to regulate the expression of these channels at the axon initial segment, which is important for the generation of action potentials. The mutant FGF14 protein fails to interact with sodium channel subunits and interferes with the interaction between wild-type FGF14 and voltage-gated sodium channel subunits in a dominant manner.16

**Spinocerebellar Ataxia Type 5**

Spinocerebellar ataxia type 5 results from mutations in the β-III spectrin gene, SPTBN2.17 Two identified mutations cause in-frame deletions of 39 and 15 base pairs, while a third missense mutation alters an amino acid in the actin/dynactin–binding region. Spinocerebellar ataxia type 5 presents clinically as a slowly progressive cerebellar syndrome typically beginning in young adults.

The disease protein β-III spectrin is highly expressed in Purkinje cells, where it stabilizes the Purkinje cell–specific glutamate transporter, excitatory amino acid transporter (EAAT4), at the plasma membrane. In SCA5 patient samples, protein levels of EAAT4 are reduced in the cerebellum. Both EAAT4 and GluRdelta2, an ionotropic glutamate receptor subunit expressed in Purkinje neurons, are reduced in the synaptosomal fractions of human SCA5 brain tissue.17 These changes suggest that ataxia in SCA5 may stem from abnormal glutamate signaling at the parallel fiber–Purkinje neuron synapse.

**Dentatorubral-Pallidoluysian Atrophy**

Dentatorubral-pallidolysian atrophy is a dominantly inherited neurodegenerative disorder characterized clinically by progressive dementia, ataxia, chorea, myoclonic epilepsy, and psychiatric disturbance. Like SCA6, DRPLA is a CAG repeat/polyglutamine disease. In DRPLA, however, the mutation alters a protein, atrophin-1, that appears to be a transcriptional corepressor.

In a recently described mouse model of DRPLA, electrophysiologic changes in various brain areas were found. The findings included age-dependent, region-specific presynaptic dysfunction of excitatory transmission in the globus pallidus, parallel fiber–Purkinje neuron synapses in the cerebellum, and decreased currents through α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and GABA_A receptors in CA1 hippocampal neurons.18 This observed reduction in Purkinje cell excitation could decrease modulation of the excitatory output of DCN neurons, leading to ataxia.

**Episodic Ataxia 6**

Mutations in the SLC1A3 gene encoding EAAT1, also known as the glial glutamate transporter, have been identified in some patients with episodic ataxia.19 This novel cause of episodic ataxia has been designated EA6.

Mutant EAAT1 has reduced glutamate uptake capacity. When coexpressed with wild-type EAAT1, mutant EAAT1 also shows reduced glutamate uptake, suggesting that it exerts a dominant-negative effect.20 Glial glutamate transporter–deficient mice have impaired motor coordination and show decreased clearance of glutamate at the parallel fiber and climbing fiber synapses in the presence of an AMPA-type glutamate receptor potentiator.21 It is intriguing to speculate that under periods of stress, defective handling of synaptic glutamate may manifest as episodic ataxia.
Paraneoplastic Cerebellar Ataxia

Cerebellar ataxia sometimes occurs in association with Lambert–Eaton myasthenic syndrome (LEMS). This syndrome is an immune-mediated disorder of the neuromuscular junction associated with antibodies directed against the voltage-gated P/Q-type calcium channel, Cav2.1. In a recent study, cerebellar symptoms were present in 9% of 97 patients with LEMS, in nearly all cases associated with small cell lung cancer.22

The fact that Cav2.1 also is implicated in hereditary ataxic disorders (SCA6, EA2) supports the view that anti-Cav2.1 antibodies underlie the ataxia in LEMS. Moreover, experimental evidence directly implicates the paraneoplastic antibody. When applied to cerebellar slices, an antibody directed against Cav2.1 reduced synaptic transmission at the parallel fiber–Purkinje cell synapse. An anti-Cav2.1 antibody also conferred an ataxic phenotype by passive transfer in mice.23

Figure 2. A schematic diagram of the stages of physiologic dysfunction and possible sites of therapy in ataxia with prominent cerebellar cortical dysfunction. Stage 1 shows normal intrinsic firing of Purkinje and deep cerebellar nucleus (DCN) neurons and their modulation, respectively, by parallel fibers and Purkinje cell (PC) output. Excitatory input is shown in green; inhibitory, red. Many degenerative ataxias traverse a stage with physiologic dysfunction (stage 2) followed by morphologic abnormalities (stage 3) prior to prominent neuronal cell loss (stage 4). Loss or dysfunction of Purkinje neurons would be expected to increase DCN excitability. Therapies aimed at improving cerebellar physiology may be effective at all stages of neurodegeneration: (1) improvement of both cerebellar cortical function and downstream DCN changes when there is Purkinje neuron dysfunction or minimal Purkinje cell loss; and (2) improvement of downstream DCN changes when Purkinje cells have died. PF indicates parallel fiber.

Physiologic Changes in Other Ataxias

Neuronal loss does not explain all of the phenotypic changes in the degenerative ataxias. Numerous studies support the hypothesis that, in earlier disease stages, neurologic symptoms primarily reflect neuronal dysfunction rather than neuronal cell loss. For example, mouse models of SCA1 show a neurodegenerative phenotype prior to significant neuronal cell loss.24 Purkinje neurons in SCA1 mice also demonstrate altered electrophysiologic properties: delayed spike onset to depolarizing current injections. In a manner similar to SCA1, physiological changes may precede neuronal loss in SCA3. Expression of expanded (pathogenic) ataxin-3 in differentiated neural cells reduced the resting membrane potential and caused a hyperpolarizing shift of the activation curve of delayed rectifier potassium currents.25 These physiologic changes preceded the onset of nuclear inclusions and ultrastructural morphological changes.
IMPLICATIONS FOR THERAPY

Most of the recognized electrophysiologic perturbations in ataxic disorders occur at the level of the cerebellar cortex. Defective synaptic input into the cerebellum through climbing, mossy fibers, or changes in Purkinje neuron intrinsic firing would be expected to result in loss of the integrative function of Purkinje neurons. A reduction in the ability of Purkinje neurons to sustain repetitive firing or a decrease in excitatory synaptic input to these neurons would decrease inhibition of the DCN, the sole excitatory output from the cerebellum. Understanding the function of the defective channels in hereditary channelopathies should facilitate the design of therapeutic agents that enhance their activity. Changes in ion channel distribution or function, even in the absence of a channel mutation, may explain symptoms in a variety of other ataxias. For these disorders, correcting aberrant physiology may be an attractive therapeutic strategy (Figure 2). For example, agents such as AMPA potentiators that could improve synaptic function at the parallel fiber–Purkinje neuron synapse might prove beneficial in the treatment of EA2, DRPLA, and SCA5. In some degenerative ataxias, the development of cerebellar symptoms may reflect a failure in DCN modulation secondary to the loss of Purkinje neurons. Loss of tonic inhibition of DCN neurons may set the intrinsic firing rate of DCN neurons too high for adequate modulation by collateral synaptic input to these neurons. Thus, agents that reduce DCN excitability could play a role in the treatment of symptoms in these currently untreatable disorders.

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