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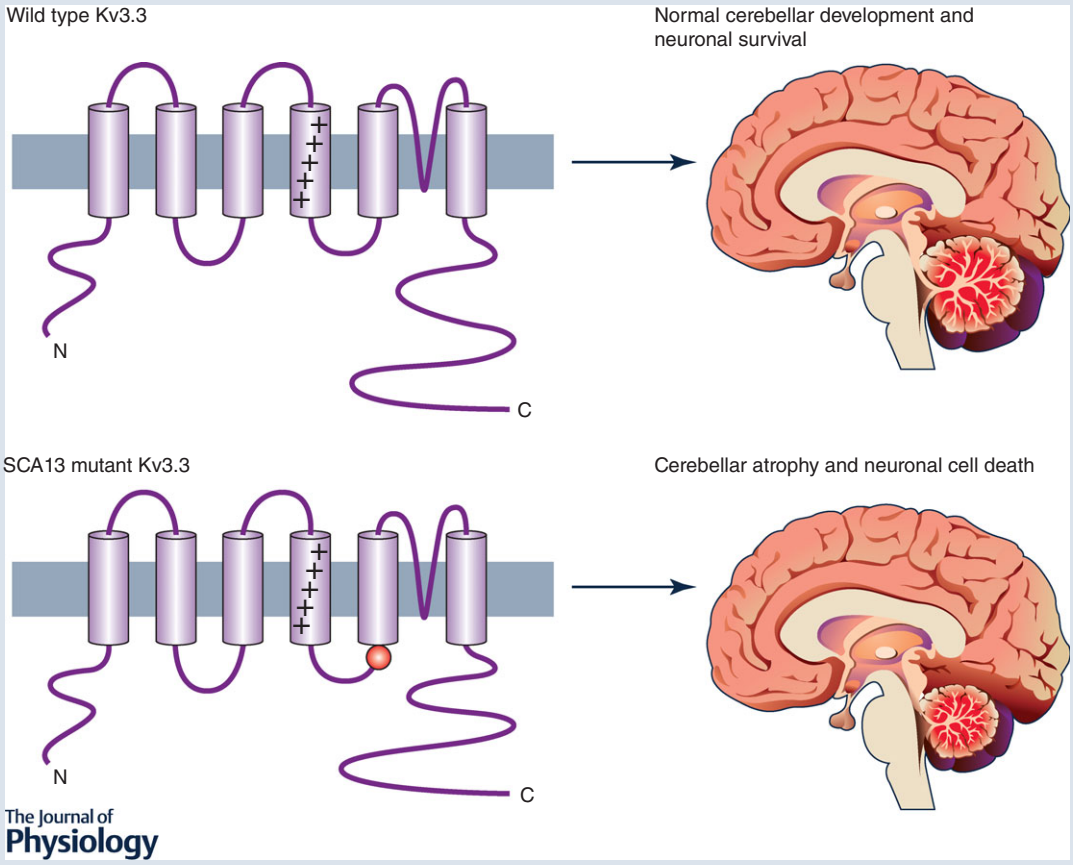
SYMPOSIUM REVIEW

Kv3.3 potassium channels and spinocerebellar ataxia

Yalan Zhang[1] and Leonard K. Kaczmarek[1,2]

[1] *Department of Pharmacology, Yale University School of Medicine, New Haven, CT, USA*
[2] *Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA*



Abstract The voltage-dependent potassium channel subunit Kv3.3 is expressed at high levels in cerebellar Purkinje cells, in auditory brainstem nuclei and in many other neurons capable of firing at high rates. In the cerebellum, it helps to shape the very characteristic complex spike of Purkinje cells. Kv3.3 differs from other closely related channels in that human mutations in the gene

Leonard Kaczmarek received his PhD from the University of London and held appointments at the University of California at Los Angeles, Université Libre de Bruxelles and Caltech before joining Yale University in 1981. He is currently Professor of Pharmacology and of Cellular and Molecular Physiology at Yale and served as Chair of the Department of Pharmacology for 9 years. **Yalan Zhang** received her PhD at the Chinese Academy of Medical Sciences and Peking Union Medical College and works at the Yale University School of Medicine as an Associate Research Scientist with Dr Kaczmarek. The major goal of the laboratory is to understand the role of potassium channels in long-term changes in neuronal excitability, as well as in neurological disorders.

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encoding Kv3.3 (*KCNC3*) result in a unique neurodegenerative disease termed spinocerebellar ataxia type 13 (SCA13). This primarily affects the cerebellum, but also results in extracerebellar symptoms. Different mutations produce either early onset SCA13, associated with delayed motor and impaired cognitive skill acquisition, or late onset SCA13, which typically produces cerebellar degeneration in middle age. This review covers the localization and physiological function of Kv3.3 in the central nervous system and how the normal function of the channel is altered by the disease-causing mutations. It also describes experimental approaches that are being used to understand how Kv3.3 mutations are linked to neuronal survival, and to develop strategies for treatment.

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Corresponding author L. K. Kaczmarek: Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520, USA. Email: leonard.kaczmarek@yale.edu

Abstract figure legend Mutations in the Kv3.3 potassium channels produce spinocerebellar ataxia type 13, a disease that results in progressive degeneration of the cerebellum.

Introduction

The Kv3.3 channel subunit is one of the four members of the Kv3 family of voltage-dependent K^+ channels (Gutman *et al.* 2005). Like the other members of this family, Kv3.1, Kv3.2 and Kv3.4, the Kv3.3 channels activate at positive membrane potentials and their major function is to drive the repolarization phase of action potentials (Rudy & McBain, 2001). While mutations in the human genes for ion channels are well known to produce disorders of excitability as well as movement disorders, it is unusual for channelopathies to cause neurodegeneration. A clear exception is spinocerebellar ataxia type 13 (SCA13), which is defined as a disease caused by mutations in Kv3.3 that result in degeneration of the cerebellum.

Spinocerebellar ataxias (SCAs) are a group of inherited diseases that result in progressive loss of motor coordination and cerebellar atrophy (Rossi *et al.* 2014). Mutations in at least 29 different genes have now been shown to result in SCAs, and these have been named SCA1–SCA29, according to the order in which they were discovered. Each of these SCAs is associated with a slightly different set of symptoms. In those cases where the disease is not manifest until middle age, patients typically retain full intellectual function but progressively lose the ability to walk and other motor functions. While the function of many of the protein products of the SCA genes is not yet understood, this is not the case for Kv3.3, whose major biological role as a channel is understood reasonably well. It remains a mystery, however, as to why mutations in this channel subunit, but not in other members of the Kv3 family are closely linked to the progressive loss of specific populations of neurons.

This review focuses on the physiological and pathophysiological role of Kv3.3, and is divided into four parts. First, we describe the properties of Kv3.3 channels and what is known of their physiological function in specific regions of the nervous system. Next we list some of the known Kv3.3 mutations that result in SCA13 and how these mutations alter channel function. We then describe what has been learned about the function of the Kv3.3 channel by studying animals in which the gene for the channel has been deleted. Finally we cover studies in which the mutant Kv3.3 genes have been expressed in animal models in an attempt to determine how these mutations alter the cellular properties of neurons and disrupt neuronal circuits.

What do Kv3.3 channels do and where are they?

Like other Kv3 family channels, the Kv3.3 voltage-dependent K^+ channel belongs to a class of ion channels sometimes termed 'high-threshold' or 'high voltage-activated' channels. These are classic delayed rectifier channels that begin to activate only at potentials more positive than ~ -20 mV. Thus they contribute very little to resting potassium conductance but are activated during the rising phase of an action potential (Rudy & McBain, 2001). Kv3.3 channels also activate and deactivate very rapidly in response to changes in voltage. Thus these channels produce very rapid repolarization of action potentials with little or no relative refractory period, allowing neurons that express these channels to fire trains of action potentials at high frequencies.

In response to a maintained depolarization, Kv3.3 channels undergo slow and partial inactivation, with a time course of several hundred milliseconds (Fernandez *et al.* 2003; Desai *et al.* 2008). This inactivation occurs through an N-type inactivation mechanism, and deletion of the cytoplasmic N-terminus of the channel eliminates inactivation. The rate of inactivation can also be regulated by activation of protein kinase C. Evidence suggests that phosphorylation of two serine residues in the Kv3.3 N-terminus prevents the N-terminal domain from producing inactivation (Desai *et al.* 2008).

As expected from their electrophysiological properties, Kv3.3 channels are expressed in neurons that fire at high

rates, particularly in the brainstem and cerebellum (Li *et al.* 2001; Chang *et al.* 2007; Puente *et al.* 2010). High levels are also found in inhibitory parvalbumin-containing cortical interneurons that typically are capable of firing at hundreds of hertz, as well as in GABA-ergic interneurons in other parts of the nervous system (Chang *et al.* 2007; Alonso-Espinaco *et al.* 2008; Nowak *et al.* 2011). Many such neurons also express the Kv3.1 channel, and the high voltage-activated channels in such neurons may be heteromeric channels containing both Kv3.3 and Kv3.1. Kv3.3 is also strongly expressed in most auditory brainstem nuclei, in which neurons may fire at rates up to 600–800 Hz to process auditory information (Li *et al.* 2001; Tong *et al.* 2010).

Because of their high levels in cerebellar Purkinje cells, a number of studies have focused on the role of Kv3.3 channels in the generation of the characteristic complex spike that is evoked in these cells by stimulation of a single action potential in the excitatory climbing fibre afferents that synapse onto their dendrites (Zagha *et al.* 2008, 2010; Veys *et al.* 2013). Kv3.3 channels are required for generation of the rapid repetitive spikelets that occur at the soma during the complex spike (Fig. 1*A*) (Zagha *et al.* 2008).

Mutations in Kv3.3 cause spinocerebellar type 13

The first genetic study demonstrating a link between human mutations in *KCNC3*, the gene encoding Kv3.3, and ataxia reported two different channel mutations: R420H, which leads to adult onset ataxia, and F448L, which results in childhood onset ataxia with cognitive delay (Waters *et al.* 2006). The R420H mutation is located in the S4 transmembrane domain that represents the major voltage sensor in these channels (Fig. 1*B*). Another mutation (R423H) has also been found in the S4 domain, but, in contrast to R420H, this produces a severe early onset spinocerebellar ataxia (Figueroa *et al.* 2010, 2011). The early onset F448L mutation is located in the S5 transmembrane region of the protein, close to the pore of the channel (Fig. 1*B*). Since that time, a number of other mutations have been found to be associated with either early or late onset spinocerebellar ataxia (Figueroa *et al.* 2010, 2011; Németh *et al.* 2013; Duarri *et al.* 2015). The early onset forms are generally associated with some intellectual disability and occasionally with seizures (Figueroa *et al.* 2011). Some of the mutants are listed in Table 1, and new mutations are still being discovered.

While the primary focus of the effects of Kv3.3 mutations has been on cerebellar function, it is evident that other neuronal circuits can be severely impacted. A clear example of this is found in the auditory system where Kv3.3 channels are found at high levels in brainstem circuits underlying sound localization (Li *et al.* 2001). Psychophysical tests have shown that, despite having normal sensitivity to sounds, individuals with the disease-inducing Kv3.3 mutations are insensitive to changes in the amplitude or timing of sounds arriving at the two ears (Middlebrooks *et al.* 2013). As a result they are incapable of determining the location of sounds in space. Interestingly, this is true for individuals who bear the mutations but have no other clinical symptoms.

A basic experimental approach to understanding how these mutations alter the function of Kv3.3 channels has been to express complementary (c)RNA encoding the mutant channels in *Xenopus* oocytes and to use two-electrode voltage clamp techniques to compare the currents with those of the wild type channels. This has revealed that at least some of the mutations result in Kv3.3 proteins that are non-functional as channels. This is the case for the R420H and R423H mutations which produce

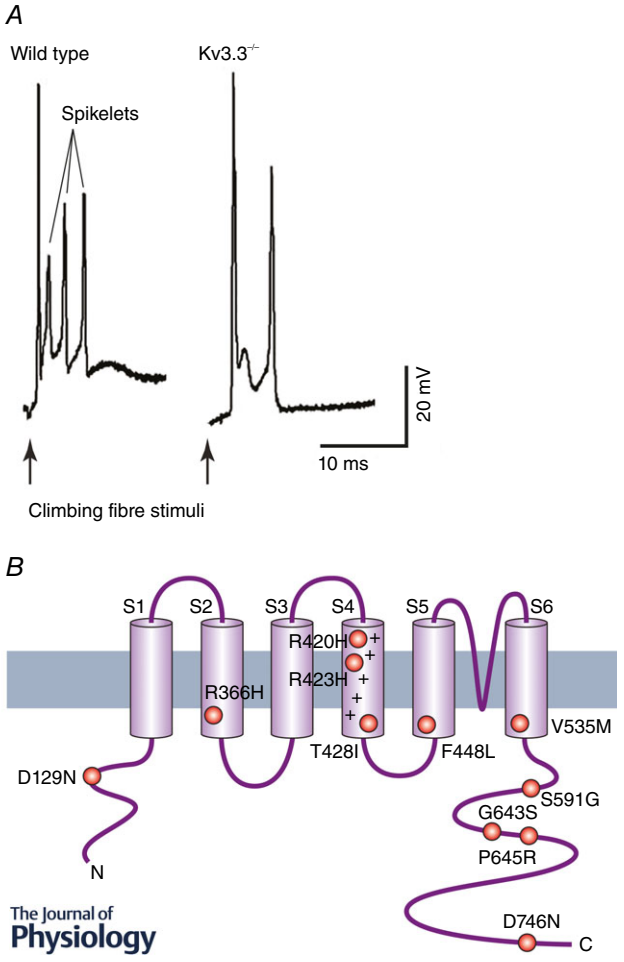

Figure 1. The Kv3.3 channel
A, examples of complex spikes evoked by stimulation of climbing fibres in cerebellar Purkinje cells from wild type and *Kv3.3*$^{-/-}$ mice (modified from Hurlock *et al.* 2008). *B*, schematic diagram indicating the locations of sites of mutations in a single Kv3.3 subunit, including mutations known to cause SCA13.

Table 1. Effects of some SCA13 mutations on properties of Kv3.3 channels

Mutation	Onset	Biophysical change	Reference
R420H	Adult	Current amplitude greatly reduced Dominant negative in heteromers with wild type Kv3.3	(Waters *et al.* 2006)
R423H	Early	Current amplitude greatly reduced Dominant negative and activation voltage shifted to negative potentials in heteromers with wild type Kv3.3	(Figueroa *et al.* 2010, 2011)
F448L	Early	Activation voltage shifted by −13 mV	(Waters *et al.* 2006; Figueroa *et al.* 2010)
S591G	Adult	Current reduced by ∼22% Activation voltage shifted by +16 mV	(Duarri *et al.* 2015)
D129N	Adult	Activation voltage shifted by −6 mV	(Duarri *et al.* 2015)
V535M	Early	Activation voltage shifted by −28 mV	(Duarri *et al.* 2015)
T428I	Early	Current amplitude greatly reduced Dominant negative and activation kinetics slowed in heteromers with wild type Kv3.3	(Németh *et al.* 2013; Parolin Schnekenberg *et al.* 2015)

no K^+ current in oocytes (Waters *et al.* 2006; Figueroa *et al.* 2010) (Fig. 2*A*). Moreover, when co-expressed with wild type Kv3.3 channels, with which they would be expected to form hetero-tetramers, R420H and R423H have a dominant-negative effect, suppressing overall current amplitude. Thus, because these are dominant mutations, one would expect SCA13 patients with these mutations to have reduced neuronal Kv3.3 current.

Biochemical experiments have provided some explanations for the reduced current observed with loss-of-function mutations such as R420H and R423H. When expressed in a mammalian cell line, these mutant channels are degraded more rapidly than wild type channels (Zhao *et al.* 2013). Levels of the mutant proteins can be increased by inhibition of the ubiquitin–proteasome pathway, treatment with a chemical chaperone, or co-expression with the Kv3.1b potassium channel, with which the mutant proteins may form heteromers (Zhao *et al.* 2013). When localized by light level or electron immunomicroscopy, the majority of R420H and R432H mutant proteins appear to be retained in the Golgi rather than being trafficked to the plasma membrane (Gallego-Iradi *et al.* 2014; Duarri *et al.* 2015). Levels of the mutant protein in the plasma membrane are reduced by over 25% relative to wild type Kv3.3 (Gallego-Iradi *et al.* 2014).

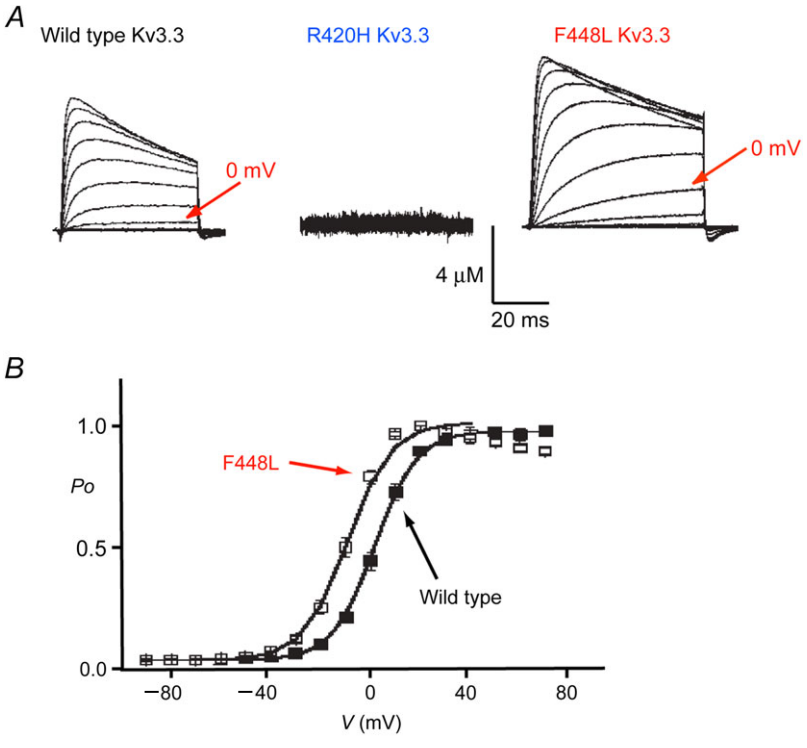

Figure 2. Effects of SCA13 mutations on Kv3.3 currents
A, examples of currents recorded under voltage clamp in *Xenopus* oocytes expressing wild type, R40H or F448L Kv3.3 channels. Arrows indicate currents evoked by a step to 0 mV. *B*, plots of estimated open probability as a function of voltage for wild type and F448L Kv3.3 channels. (Modified from Waters *et al.* 2006.)

In contrast to loss-of-function mutations such as R420H and R423H, other disease-causing mutations, such as the early onset F448L mutation, result in fully functional Kv3.3 channels that make it to the plasma membrane. The electrophysiological properties of these channels, however, differ slightly from those of wild type Kv3.3 (Waters *et al.* 2006; Duarri *et al.* 2015) (Table 1). For example, the voltage dependence of activation of F448L channels is shifted toward more negative potentials (by ∼13 mV, Fig. 2*A* and *B*) and the rate at which the channels close following deactivation is slower than that of wild type Kv3.3 (Waters *et al.* 2006). Both of these factors would be expected to increase rather than decrease the amount of K^+ current evoked by physiological depolarizations.

How can it be that a loss of Kv3.3 current, as in the R420H and R423H mutations, and an increase in K^+ current, as with the F448L mutation, both produce spinocerebellar ataxia? Moreover, how is it that one loss of function mutation (R420H) produces the adult onset ataxia while the other (R423H) produces the more devastating early onset disease? One hypothesis, supported by experimental evidence, is that loss of current alone leads to the late onset disease, while changes in kinetic behaviour and voltage dependence are required to produce early cerebellar degeneration (Minassian *et al.* 2012). The Kv3.3 channel in most SCA13 patients is likely to be a tetramer containing both wild type and mutant channels. The Papazian laboratory co-expressed the late onset R420H mutant with wild type Kv3.3 channels. They found that, although R420H reduced overall current amplitude, the kinetic behaviour of the heteromeric channels was identical to that of homomeric wild type Kv3.3 channels. In contrast, the voltage dependence of the current that results from heteromeric R423H/wild type channels was found to be shifted to negative potentials. In this respect, the R423H/wild type heteromeric channels resemble F448L mutant channels, which also cause the early onset disease. Moreover, in addition to the altered voltage dependence, the kinetic behaviour of the R423H/wild type heteromers was also different from that of wild type Kv3.3 homomers (Minassian *et al.* 2012).

Lessons from Kv3.3 knockout animals

Because some of the Kv3.3 mutations that lead to SCA13 result in partial loss of Kv3.3 current, one might expect complete loss of all Kv3.3 channels to severely affect motor function and that knockout of the *Kv3.3* gene could provide an animal model of the disease. Surprisingly, however, the phenotype of *Kv3.3*$^{-/-}$ mice is relatively mild. Only by quantification of movements can one convincingly detect a difference between *Kv3.3*$^{-/-}$ and wild type animals. Their gait is altered in that they make increased lateral deviations while walking (Joho *et al.* 2006). They are more likely to slip while walking on a narrow beam (Joho *et al.* 2006; Hurlock *et al.* 2008). They are also prone to spontaneous muscle twitches. The learning of motor behaviours, however, appears normal in *Kv3.3*$^{-/-}$ animals (Hurlock *et al.* 2009).

Kv3.3 channels are expressed in both the somata and the dendrites of cerebellar Purkinje cells. Consistent with a role for Kv3.3 in shaping the firing patterns of these neurons, Purkinje cell action potentials are broader in *Kv3.3*$^{-/-}$ mice than in wild type animals (Hurlock *et al.* 2008). In addition the Purkinje cells have fewer complex spikes and a reduced frequency of spikelets (Fig. 1*A*). These electrophysiological changes can be rescued by re-introducing wild type Kv3.3 channels selectively into Purkinje cells of *Kv3.3*$^{-/-}$ animals, but this does not rescue the motor behaviours (Hurlock *et al.* 2008). The excitability of the distal dendrites, and the amplitude of cytoplasmic Ca^{2+} signals evoked by stimulation of the dendrites, is also increased in Purkinje cells of *Kv3.3*$^{-/-}$ knockout mice (Zagha *et al.* 2010).

A much more severe motor phenotype is observed when the *Kv3.3* gene is deleted in combination with that for the closely related channel Kv3.1 (Espinosa *et al.* 2001, 2004). Such double knockout animals have severe ataxia as well as other disorders of movement. They are constitutively hyperactive, appear unbalanced when moving, and undergo whole-body jerks every few seconds (Espinosa *et al.* 2004). Electrophysiological studies indicate that a variety of synaptic pathways are altered by the deletion of both *Kv3.3* and *Kv3.1* genes (Matsukawa *et al.* 2003; McMahon *et al.* 2004; Hurlock *et al.* 2009). Like the *Kv3.3*$^{-/-}$ mice, however, these animals appear to have no learning or memory deficits (Espinosa *et al.* 2001).

Perhaps surprisingly, in light of the cerebellar degeneration that is associated with SCA13, complete loss of either the Kv3.3 or the Kv3.1 channel, or elimination of both channels together, has not been reported to produce neurodegeneration in any class of neurons.

Animal models of SCA13

A mouse model of SCA13 would require construction of a heterozygous strain that expresses both wild type and mutant Kv3.3 channels. While there have been no reports of such a knock-in mouse model to date, it is likely that such strains will shortly be available. A study of how the R423H Kv3.3 mutation alters the physiology of Purkinje cells has, however, been provided by using a lentivirus to introduce this mutant into cerebellar neurons in primary cell culture (Irie *et al.* 2014). As with human Kv3.3, the introduction of the corresponding mutation into the mouse channel (R424 in the mouse numbering – for simplicity we will retain the human numbering) causes a dominant-negative effect, suppressing current amplitude but also shifting the voltage dependence and

kinetic behaviour of the heteromeric wild type/mutant channels. The rate of inactivation of the heteromeric channels during sustained depolarization was also found to be significantly increased over that in wild type homomeric channels (Irie *et al.* 2014).

In part, the electrophysiological effects of introducing the R423H mutation into Purkinje cells in culture resembled those previously reported for cells from *Kv3.3*$^{-/-}$ animals. The cultured neurons had a lower density of voltage-dependent K^+ current, broadened action potentials and elevated intracellular Ca^{2+} levels (Irie *et al.* 2014). In addition, however, the mutant channels also produced more profound cell biology changes. Purkinje cells expressing the mutant Kv3.3 became smaller in size than control cells, and their dendrites became shorter and less elaborate. Finally, in such cultures, the density of Purkinje cells declines over a period of 10 days, but the density of cells expressing the mutant channels declined more rapidly than that of control Purkinje cells, suggesting that the mutation increases the rate of cell death (Irie *et al.* 2014).

Granule cells, the other major neuronal type in the cerebellum, do not express Kv3.3. The introduction of the R423H mutant channels into granule cells had no effect on their survival, suggesting that R423H proteins do little or nothing by themselves, and that functional heteromers containing wild type Kv3.3 are required to produce cell death (Irie *et al.* 2014). In the Purkinje cell, the effects on cell survival and dendritic morphology could be partially rescued by treatment with a blocker of P/Q-type voltage-dependent Ca^{2+} channels, suggesting that the elevated intracellular Ca^{2+} levels found in cells expressing the mutant channels contribute to the morphological changes and to cell death (Irie *et al.* 2014).

In trying to understand a complex biological effect it is often useful to study a simpler biological system. Experiments with zebrafish have provided many insights into neuronal development (Grunwald & Eisen, 2002), particularly that of motor neurons as well as of the cerebellum and cerebellar Purkinje cells (Hsieh *et al.* 2014). A series of studies using zebrafish have provided insights into the different effects of early onset and adult onset SCA13 mutations (Mock *et al.* 2010; Issa *et al.* 2011, 2012). As in mammals, Kv3.3 channels in zebrafish activate and deactivate rapidly (Mock *et al.* 2010) to allow neurons to fire action potentials repetitively at high rates. Biophysical characteristics such as slow N-type inactivation are also conserved. Importantly, mutations corresponding to the adult onset R420H and early onset F448L SCA13 mutations have the same effect on channel behaviour as in the mammalian channels. The former suppresses Kv3.3 activity by a dominant-negative mechanism while the latter shifts the voltage range of activation to negative potentials and slows deactivation (Mock *et al.* 2010).

Fast-spiking motor neurons in the spinal cord of zebrafish express Kv3.3 (Issa *et al.* 2011). Introduction of the human dominant-negative R420H mutant subunit decreased the K^+ current, as well as the excitability of these neurons. It also decreased the behavioural startle response that is mediated by these cells (Issa *et al.* 2011). Expression of the zebrafish mutant channel corresponding to this late onset mutation had, however, only minor effects on the development of an identified motor neuron (termed CaP), increasing the complexity of its distal axonal arbor (Issa *et al.* 2012). In sharp contrast, expression of a zebrafish channel corresponding to the early onset F448L human mutation caused the axons of CaP neurons to make many pathfinding errors, and to send axons into regions normally innervated exclusively by other identified motor neurons (Issa *et al.* 2012).

Conclusions

Neurodegeneration can be triggered by abnormalities in processes such as aggregation of cytoplasmic proteins or in mitochondrial pathways linked to cell fate and survival. A key question that has yet to be answered, however, is how changes in the function of an ion channel lead to cell death. One possibility, supported by experiments covered in this review, is that altered neuronal firing patterns lead to the abnormal accumulation of intracellular Ca^{2+}, resulting in neurotoxicity (Irie *et al.* 2014). Another possibility is that the formation of inappropriate axonal or dendritic connections triggers cell death during brain development (Issa *et al.* 2012). While these may be important contributory factors, they raise the question of why cerebellar neurodegeneration is selectively produced by mutations in Kv3.3 but apparently not in other channels. A wide variety of channelopathies caused by mutations in other K^+ channels have been described, including the close family member Kv3.1 (Muona *et al.* 2015), and these generally cause disorders of excitability such as epilepsy. While it is entirely possible that such mutations in other channels also influence cell survival, channelopathies have not been generally associated with neurodegeneration.

Another factor that may be important in considering how Kv3.3 channels influence the development and cellular properties of neurons is that the Kv3.3 subunit is a much larger protein than other channels in the Kv3 family. This can be attributed to a much longer cytoplasmic C-terminal domain. The biological function of this longer cytoplasmic domain is not yet fully understood, but, as has been found for other channels (Kaczmarek, 2006; Lee *et al.* 2014), it may participate in protein–protein interactions with cytoplasmic signalling pathways. It is therefore possible that SCA13 mutations ultimately influence these cytoplasmic interactions, leading to events that cannot be predicted by changes in excitability alone.

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Additional information

Competing interests

None declared.

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