

Development of an assay for inhibitors of the deleterious interaction between wild type and Episodic ataxia-2 mutant Ca_v2.1 channels

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Scientific summary

Episodic ataxia-2 (EA2) is caused by mutations in the gene *CACNA1A*, encoding the voltage-gated calcium channel Ca_v2.1. Many mutations predict the formation of a truncated channel protein, although some are missense (Jen,2008). Evidence suggests that the deleterious effects of these mutations cannot solely be due to haplo-insufficiency caused by loss or reduced function of the mutant channel. Therefore, there may be a dominant-negative effect of the mutant channel (Jouvenceau et al.,2001; Page et al.,2004; Jeng et al.,2006). There is evidence that interaction occurs between the wild-type and mutant channels, resulting in misfolding and aggregation, triggering cellular protective mechanisms, including the unfolded-protein response (Page et al.,2004) and endoplasmic reticulum-mediated decay of the wild-type channels (Mezghrani et al.,2008).

We have recently found that a domain in the free N-terminus of the EA2 mutant channels is sufficient to trigger this dominant-negative effect (Page et al.,2010), which we feel represents a significant scientific advance. If this deleterious interaction between EA2 mutant and wild-type Ca_v2.1 channels could be prevented, this might represent a potential therapeutic avenue.

We therefore propose to examine whether we can prevent the interaction by using small peptides mimicking the N-terminal interaction site. To do this, we will develop fluorescence-based assay to rapidly assess the dominant-negative effect of the EA2 mutant channels on cell-surface expression of the wild-type channels, and we will then be able to examine the effect of co-expression of peptides or application of cell-permeant peptides.

References:

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Lay summary

Episodic ataxia-2 (EA2) is a genetic condition that particularly affects the function of a part of the brain called the cerebellum, which is involved in the coordination of movement. The gene that is affected in EA2 is called *CACNA1A*, which codes for a protein that is very important in cerebellar function. This particular protein is called a calcium channel. EA2 is a dominant disease, meaning you only need a mutation in one of your two copies of this gene to have the disease. This implies either that one copy of the gene does not produce enough normal protein or that the mutant protein is in some way deleterious. We and others have found previously that some EA2 mutant channels interact with the normal channels and cause them to be broken down, and also activate cellular stress processes. This may mean that there are fewer normal channels than expected to allow brain cells in the cerebellum to function as they are supposed to. In addition, aggregation of proteins is generally detrimental to cells, and has been found to be the basis for several neurodegenerative conditions, including Alzheimer's disease. It may be the reason why some people with EA2 are found gradually to lose brain cells in the cerebellum.

We have already found which part of the EA2 mutant and normal calcium channels is responsible for this potentially toxic interaction, so we want to design molecules that will prevent the interaction, and design a test that will let us find out more rapidly if they are effective. All this work will be done in cells grown in our laboratory, because we need to first obtain proof in a simple system as to whether it will work. We hope to be able to do this within 1 year. We could then go on to examine whether it might be effective in more complex systems, but we will do this at a later stage, as long as results from this study look promising.

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