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PERSPECTIVE

The inositol metabolism pathway as a target for neuroprotective strategies

Neurodegeneration is characterized by the progressive and permanent loss of neurons. Degeneration typically results in a debilitating loss of function in an otherwise healthy person. Neurodegenerative diseases have enormous direct health care costs, with some estimates for diseases, such as Alzheimer's disease exceeding $36,000 USD per patient annually. Currently there is a lack of effective treatments for neurodegenerative disease, thus there is no way to slow or prevent the irreversible death of neurons in patients suffering from these diseases. Growing evidence suggests that the pathways controlling the levels of intracellular calcium [Ca^{2+}], including the second messenger inositol 1,4,5-trisphosphate ($InsP_3$), are disrupted in some of the more common forms of neurodegeneration. Dysfunction in these pathways allow for excessive and toxic levels of intracellular [Ca^{2+}] to accumulate. One possible neuroprotective strategy would be to target $InsP_3$ regulatory pathways to prevent excess calcium release from intracellular stores. This review will focus on the implication of $InsP_3$ pathways in current strategies of neuroprotection. While the causes of neurodegeneration are diverse, common pathological pathways may exist between diseases and protective targeting of a common pathway would have the potential to treat genetically distinct diseases.

The role of soluble inositol phosphatases in the regulation of Ca^{2+}: Soluble inositol phosphates (Ins) and membrane-bound phosphatidylinositols (PtdIns) control a wide variety of cellular processes. The critical nature of these pathways in terms of cellular function and survival becomes evident when the proteins that comprise these pathways are disrupted. Several kinase and phosphatase enzymes form pathways that precisely control their Ins/PtdIns substrates. The enzymatic steps are comprised of large gene families, *e.g.*, in human there are 10 members in the inositol 5-phosphatase family. The membrane bound PtdIns have diverse activities, *e.g.*, regulation of endosomal traffic and controlling the phosphorylation of Akt, while the soluble Ins pathway in neurons is critical to the regulation of intracellular [Ca^{2+}]. The central mechanism controlling Ca^{2+} release from intracellular stores involves the generation of inositol 1,4,5-trisphosphate ($InsP_3$) in response to an extracellular signal. This pathway is well defined in the cerebellar Purkinje cell, in part because disruption of these genes results in readily identifiable ataxic disorders. In Purkinje cells an excitatory glutamate signal from the cerebellar parallel fibers is transduced through metabotropic glutamate receptor 1 (mGluR1). This causes the generation of $InsP_3$, which then binds inositol 1,4,5-triphosphate receptor 1 (ITPR1) and causes the release of Ca^{2+} (Berridge et al., 2003). The $InsP_3$ molecule is then broken down to Ins through a series of Ins phosphatases. This pathway has been well defined in the non-disease state, more recently a number of studies have also characterized the phenotypic results of disruption of these genes at the systems level, specifically in neurological disorders.

Mutations in CNS expressed $InsP_3$ pathway genes cause neurological disorders: Several of the genes involved in producing, responding to, and the breakdown of $InsP_3$ are mutated in both human neurological disease and their counterpart mouse models. At least seven ataxic mouse mutants are listed on the Mouse Genome Informatics database that cluster in this pathway including: *mGluR1*, *Gnaq*, *Plcb4*, *Itpr1*, *Inpp4a*, *Inpp5a* and *Car8*. These phenotypes indicate that Ca^{2+} release *via* ITPR1 is critical for the proper function of Purkinje cells, the sole output neuron of the cerebellum. Interestingly, while all these mutants have an ataxic phenotype, only the *Inpp4a* and *Inpp5a* mutants, which are downstream of ITPR1 activation, show Purkinje cell degeneration. While many of these genes have been identified in association with mouse model ataxia, evidence suggests that this pathway is also associated with human ataxia. Mutations in *Inpp4a* have been described in mouse model and human degenerative ataxia. The mouse mutant is characterized by lethality near wean and perinatal neurodegeneration in Purkinje cells and CA1 region of the hippocampus. *Inpp4a* is expressed at high levels in Purkinje cells, and moderate levels throughout the brain. The mutation was identified as a single base deletion that resulted in a frameshift; however, due to the fact no mRNA could be detected, the mutation was considered a null (Nystuen et al., 2001). A similar phenotype was identified in humans. A 1.7 kb deletion in *INPP4A* was identified in a consanguineous family (Sheffer et al., 2015). The patient had early onset cerebellar atrophy and notably, myoclonic seizures. The mutant mouse model showed histological evidence of seizures in terms of the typical hippocampus CA1 region degeneration. Therefore, it appears that in this case the mouse is an accurate model of the human disease. The *Inpp5a* mutant mouse is characterized by perinatal lethality, but a subset of mutants survive into adulthood. In these mutants, a progressive Purkinje cell degeneration and ataxia is evident (Yang et al., 2015). There are no reports of *Inpp5a* being linked to a human disease; however, it is an excellent candidate gene for human ataxic disorders. Given the heterogeneous nature of sporadic ataxias and their difficultly in differential diagnosis, it would be greatly beneficial to identify additional genes to be included in genetic tests.

The involvement of misregulation of intracellular calcium in neurodegeneration: While the genetic mutations involved in neurodegeneration are diverse, evidence shows that some commonality may exist in their molecular pathological mechanisms. Disruption of the finely controlled levels of intracellular [Ca^{2+}] have been implicated in excitotoxic neuron death, and excessively high levels promote necrosis. The pathways excessive [Ca^{2+}]$_i$ disrupt are partially understood, one potential disease mechanism would involve the increased oxidative stress from excessive [Ca^{2+}]$_i$ taken up by the mitochondria leading to the activation of apoptotic pathways. Several genetically distinct diseases have been linked to dysfunction of ITPR1. For example, the expanded form of the Huntington disease protein was found to interact with ITPR1, and it was shown that this interaction causes elevation of [Ca^{2+}]$_i$ (Tang et al., 2005). A similar effect is observed with the expanded form of ATX2 (Atx2-58Q), which interacts with ITPR1 and leads to greater release of Ca^{2+} from the endoplasmic reticulum when induced (Liu et al., 2009). Considered together these examples suggest that the disruption of Ca^{2+} release through ITPR1 may be a pathological neurodegenerative mechanism and that the regulation of $InsP_3$ to Ins may be a viable therapeutic target.

Neuroprotective strategies that target $InsP_3$ metabolism: Currently, there is a lack of effective treatments for neurodegenerative disease; thus, there is no way to slow or prevent the irreversible death of neurons in patients suffering from these disorders. The goal of much research is to identify neuroprotective treatments for degenerative conditions. One such strategy involves limiting $InsP_3$ concentration by expressing the Ins phosphatase enzymes that breakdown the $InsP_3$ signal. Recent evidence in a mouse model of spinocerebellar ataxia 2 (SCA2) has demonstrated that this strategy is viable. The AAV driven expression of a modified, less active *INPP5A* (R343A) significantly reduced Purkinje cell degeneration and improved motor function in SCA2 mutant mice; however, motor function was decreased in AAV treated normal mice, thus demonstrating the need for fine control (Kasumu et al., 2012). A similar effect was shown with lithium, a known inhibitor of two InsP phosphatase enzymes. Lithium was shown to partially rescue function in the SCA1 mouse model and attenuate pathology (Watase et al., 2007). Taken together these data are proof of concept that it is possible to prevent neurodegeneration by modulating the levels of $InsP_3$. However, it appears that dosing will be complicated to ensure $InsP_3$ concentration does not

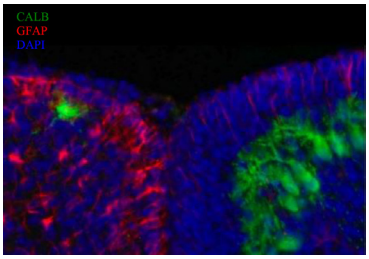

Figure 1 Patterned neuroprotection in *Inpp4a* mutant mice.
Calbindin immunostaining shows surviving Purkinje cells (green) in areas of the cerebellum. In other areas Purkinje cells are lost or dying and high levels of GFAP (red) are present indicating gliosis. This protection correlated to the expression of EAAT4. DAPI: 4′,6-diamidino-2-phenylindole; EAAT4: excitatory amino acid transporter; GFAP: glial fibrillary acidic protein.

reach excessive levels where it causes toxic activation of ITPR1 nor levels that are too low to properly activate ITPR1.

Another neuroprotective strategy would be to partially inhibit the extracellular glutamate signal thereby reducing the generation of intracellular $InsP_3$. In both the *Inpp4a* and *Inpp5a* mutant mouse cerebellum, consistent areas of healthy Purkinje cells are observed in patches that correspond to the compartmentalized expression pattern of *Eaat4* (**Figure 1**) (Sachs et al., 2009). EAAT4 is a glutamate transporter that clears the synapse of glutamate and dampens the activation of mGluR1 likely decreasing $InsP_3$ production. EAAT4 is expressed in groups of adjacent Purkinje cells. When viewed coronally, the expression pattern of Eaat4 appears in stripes of on and off expression that transverse the folia of the cerebellum. In the *Inpp4a* and *Inpp5a* mutant, in the areas where EAAT4 expression is lacking, complete Purkinje cell loss is observed whereas Eaat4 expression is preserved in surviving Purkinje cells. Therefore, while this data is correlative, given the function of EAAT4 it is likely that clearance of glutamate from the synapse dampens the potential of the neuron and is protective.

Still other neuroprotective strategies would modulate intracellular Ca^{2+} regardless of $InsP_3$ by inhibition of the Ca^{2+} channels. For example, CNTF is a neurotrophic factor that has demonstrated neuroprotective qualities in the retina; however, the mechanism of action has been largely unclear (Wen et al., 2012). Recent data has shown that CNTF has a dramatic effect in reducing the expression of CNGA1 and CNGB1, proteins that control Ca^{2+} influx into the neuron (Komaromy et al., 2013). Reduction of the overall number of CNGA1 and CNGB1 channels by CNTF may reduce the potential for $[Ca^{2+}]_i$-induced excitotoxicity. This is supported by studies showing that a direct knockdown of CNGA1 by shRNA is protective of photoreceptors in the *Pde6b* mouse model of retinitis pigmentosa, while at the same time reducing the maximal ERG response (Tosi et al., 2011). Similar to the effect that AAV-Inpp5a had on wild-type mice, a reduction in dark-adapted ERG amplitudes was observed in shRNA-Cnga1 treated normal mice compared to control.

Another potential neuroprotective strategy would be to target the downstream effects of excessive Ca^{2+}, such as the induction of apoptosis. The JNKs, c-Jun N-terminal protein kinases, are a family of genes originally described due to their activation following environmental stresses. Activated JNKs promote the apoptosis of neurons. Due to the central role JNK plays in apoptosis, inhibition of this pathway has a potential as a neuroprotective therapeutic intervention (Antoniou et al., 2011). Following the acute application of kainic acid, seizure activity, neuronal apoptosis and phosphorylation of c-Jun was significantly reduced in *Jnk3* null mice compared to wild-type control (Yang et al., 1997). However, this effect has failed to be observed in other degenerative models. In our studies we determined that apoptosis in the *Inpp4a* mutant hippocampus was promoted by JNK3 and its absence significantly modified the *Inpp4a* mutant phenotype in a dose-dependant manner. Compound mutant mice were still ataxic due to the cerebellar defect; however, general vigor and lifespan were improved. Histological analysis demonstrated that the neurodegeneration in the hippocampus occurred, albeit the onset was delayed (our unpublished data). These results suggest that inhibition of excitotoxic apoptosis is a potential therapeutic strategy; however, with chronic insults, the neuron may degenerate by other pathways.

While the causes of neurodegeneration are diverse, some commonality may exist in their molecular pathological mechanisms. The pathways controlling the levels of intracellular calcium $[Ca^{2+}]$ are primarily disrupted in some of the more common neurodegenerative disease and may provide an attractive target for therapeutic intervention. Proof of concept studies have shown that manipulation of the pathways surrounding ITPR1 activation protect neurons from degeneration; however, further studies are necessary to determine the feasibility of this therapeutic approach.

Arne M. Nystuen*, Andy W. Yang

Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE, USA (Nystuen AM, Yang AW)
Currently at Neurotech, Inc., Cumberland, RI, USA (Nystuen AM)

***Correspondence to:** Arne M. Nystuen, Ph.D., arne.nystuen@gmail.com.*
***Accepted:** 2015-10-26*
***orcid:** 0000-0002-1433-3661 (Arne M. Nystuen)*
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