Determining malfunction in the neuron or muscle within neuromuscular dysfunction stemming from adenylosuccinate lyase deficiency

Chantel Hennings, McNair Scholar
The Pennsylvania State University

McNair Faculty Research Adviser:
Wendy Hanna-Rose, Ph.D.
Department Head and Professor
Biochemistry and Molecular Biology
Eberly College of Science
The Pennsylvania State University

Abstract

Adenylosuccinate lyase (ADSL) is a bi-functional biosynthesic enzyme for purine metabolism. The loss of the function in this enzyme causes a rare and often misdiagnosed disorder that ranges in severity. In humans, the severity ranges from fatal to phenotypes such as epilepsy, muscle dysfunction, and autistic like symptoms. *Caenorhabditis elegans* share the purine metabolic pathway with humans as well as showing a similar neuromuscular phenotype with a dysfunction in ADSL. To determine the question of whether the dysfunction is because of loss in the neuron or the muscle we used transgenic *C. elegans* with an extra chromosome that reestablishes the ADSL function in either the neuron or the muscle. Aldicarb was used to test the neuron and levamisole was used to test the muscle. The muscular transgenic C. elegans reacted like typical *adsl-1* in aldicarb, indicating an issue with the muscle since neural transgenic behaved more like normal. While the results with levamisole were inconclusive and will need to be adjusted in future experiments.

Introduction

Adenylosuccinate lyase (ADSL) deficiency is a recessive neurological disorder that causes a fault in the synthesis of purines through the de novo pathway which impinges on purine nucleotide recycling pathways. In humans, the primary function to be affected is neuromuscular with varying severity and onset stages. The most extreme cases are neonatal encephalopathy resulting in death just a few weeks after birth stemming from respiratory failure, seizures, and absences of spontaneous movement. Later onsets symptoms are seizures, ataxia, cognitive dysfunction, and autistic like behaviors. The only way to diagnose is through spinal fluid or urine by examining the accumulation levels of succinylaminoimidazole carboxamide riboside (SAICAr) and succinyladenosine (SAdo) (Macchiaiolo).

Adenylosuccinate lyase Deficiency

In order to learn if the dysfunction is in the neuron or the muscle, *Caenorhabditis elegans* are studied because purine metabolic pathways are conserved within all eukaryotes, and they have a well-characterized nervous system that is crucial for exploring the symptomatic traits of ADSL (Kappock; White). Neuromuscular deficiency will need to be explored to determine if the issue is within the neuron or the muscle.

Effects of Drugs on Neuromuscular Junction in *C. elegans*
The neuron releases the chemical acetylcholine (ACH) into the synapse where it is broken down through hydrolyzation into acetate and choline by the enzyme acetylcholinesterase (Mahoney). In the presence of an inhibitor like Aldicarb, ACH is no longer metabolized, causing a buildup in the synapses and in turn causing an over stimulus of choline receptors on the muscle leading to constant contraction. Thus, Aldicarb causes a paralysis in the wild type. It indicates an issue within the neuron if the *C. elegans* has delayed response (Mahoney). Whereas, in the presence of a cholinergic agonist like Levamisole, acetylcholinesterase is not hindered. Levamisole imitates ACH to appear as if there is an overproduction in the synapsis to overload the receptors on the muscle. If delayed response to levamisole occurs, then it shows a defect within the muscle, which is most likely to be caused by the receptor's inability to function (Lewis).

The Hanna-Rose lab at Pennsylvania State University uses both Aldicarb and Levamisole to study the effects they have on *C. elegans*. Using normal as the control (N2) and ADSL deficient (*adsl-1*) animals at the experimental strain it appeared that the muscle was malfunctioning because the *adsl-1* animal reacted with delayed paralysis for both chemicals aldicarb and levamisole. I will use transgenic animals to further investigate where the greater defect lies. There will be three different types of transgenic animals: 1) *adsl-1* with the ADSL function restored in the neuron 2) *adsl-1* with ADSL function restored in the muscle and 3) *adsl-1* with no function restored. These will be tested alongside N2 and *adsl-1* animals for added controls. My overall hypothesis is that ADSL function is important in the muscle. Thus, I have two sub-hypotheses, one for each type of transgenic *C. elegans* with both relating to the muscle function. First, if ADSL is only restored in the neuron, then the muscle will remain dysfunctional. Second, if ADSL is expressed only in the muscle, then the muscle function will be restored.

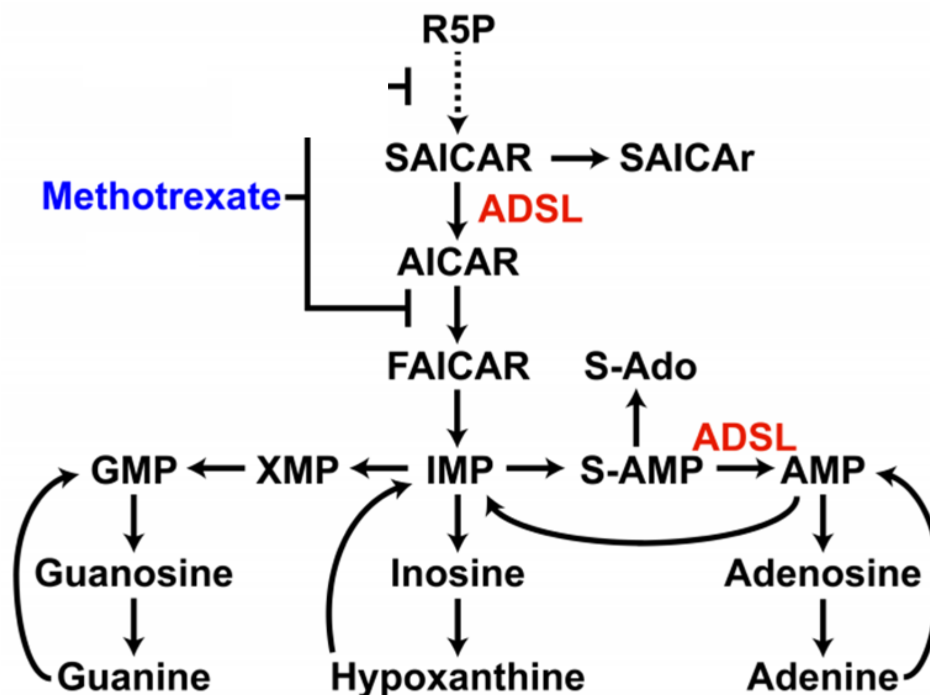

Figure 1: Purine Metabolic Pathway. ADSL (marked in red) functions twice in de novo purine biosynthesis. Abbreviations: SAICAR, succinylaminoimidazole carboxamide ribotide; SAICAr, succinylaminoimidazole carboxamide riboside; ADSL, adenylosuccinate lyase.

Methods

C. elegans strains and maintenance

The controls were N2, *adsl-1* (tm3328)/hT2, and HV856. The strain HV856 also doubled as the balancer for the transgenic animals. The balancer act as a visible marker though GFP in the pharynx, inhibits recombination, and is recessive homozygous lethal. (McKim). There are two different sets of transgenic *C. elegans* being studied: 1) *adsl-1* with ADSL function restored in the muscle strains: HV920, HV923, HV925 (Table 1); and 2) *adsl-1* with the ADSL function restored in the neuron strains: HV932, HV933, and HV934 (Table 1). All strains were grown on NGM agar plates with 50 µl of the food source OP50 *Escherichia coli* and stored at 20ºC (Brenner). To avoid the scarcity of *adsl-1* in one progeny due to *adsl-1* being autosomal recessive, *C. elegans* were maintained on 100 x 15 mm plates with three equal distance 50 µl of food spotted.

Table 1. C. elegans experimental strain descriptions

Strain	Genotype	Extrachromosomal Array
HV856	*adsl-1(tm3328)*/hT2 l; psEx306	[50 ng/µl pTG96 (sur-5p::GFP) + 50 ng/µl p Bluescript]
HV920	*adsl-1(tm3328)*/hT2 l; psEx372	[10 ng/µl pAA1 (myo-3p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]
HV923	*adsl-1 (tm3328)*/hT2 l; psEx375	[10 ng/µl pAA1 (myo-3p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]
HV925	*adsl-1 (tm3328)*/hT2 l; psEx377	[10 ng/µl pAA1 (myo-3p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]
HV932	*adsl-1 (tm3328)*/hT2 l; psEx384	[10 ng/µl pAA2 (unc-14p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]
HV933	*adsl-1(tm3328*/hT2 l; psEx385	[10 ng/µl pAA2 (unc-14p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]
HV934	*adsl-1 (tm3328)*/hT2 l; psEx386	[10 ng/µl pAA2 (unc-14p::*adsl-1*::GFP) + 50 ng/µl pTG96 (sur-5p::GFP) + 40 ng/µl pBluescript]

Paralysis Assays

Unspotted NGM plates were treated with either aldicarb or levamisole to have a final concentration of 0.5mM or 1mM respectively at the beginning of the week. This was done by placing 8mL of NGM on the plate with a stock concentration of 10mL of either chemical, aldicarb or levamisole where both were diluted in 70% ethanol. After a day of being set out at room temperature to dry, the treated plates were spotted with 10 µl of OP50 *E. coli* to help localize the *C. elegans* to the center of the plate for easier monitoring. Once the food has dried, the plates were stored in 4ºC until needed. Roughly 10 hermaphrodites of each strain were cultured for a day in 20ºC. Then each genotype was placed on a separate treated plates and observed every 30 minutes for 5 hours or until all stains were fully paralyzed (Mahoney).

Results and Discussion

Aldicarb

NGM plates were treated with aldicarb then 10µl OP50 *E. coli* was spotted at the center. The first sub hypothesis stated if ADSL is only restored in the neuron, then the muscle will remain dysfunctional. *adsl-1* and HV856, a transgenic stain with no restored ADSL function to set a more established control than plain *adsl-1* both fully paralyzed at 60 minutes standing as a marker for typical ADSL dysfunction. In contrast, N2 fully paralyzed at 240 minutes to indicate normal behavior (figure 2). *adsl-1* mutants are more sensitive to aldicarb and paralyze faster. We expect muscular transgenic *C. elegans* to behave like *adsl-1* if function is restored to a normal state in the neural transgenic.

Neural Transgenic

For the neural transgenics, HV932 behaved as *adsl-1*, HV933 behaved closer to *adsl-1* but fully paralyzed 30 minutes after, HV934 fully paralyzed halfway between *adsl-1* and N2 (Figure 2). Because neural transgenes restored some function to *adsl-1* making them more like N2, it appears that function in neuron is important for the role that ADSL plays in aldicarb response.

Muscular Transgenic

For the muscular transgenics, HV920 behaved as *adsl-1* while both HV923 and HV925 behaved like *adsl-1* by paralyzing 30 minutes after (Figure 2). Since nearly all the strains behaved the same or close to the ADSL deficient strains, there is no evidence that response to aldicarb is affected by expression of ADSL in muscle.

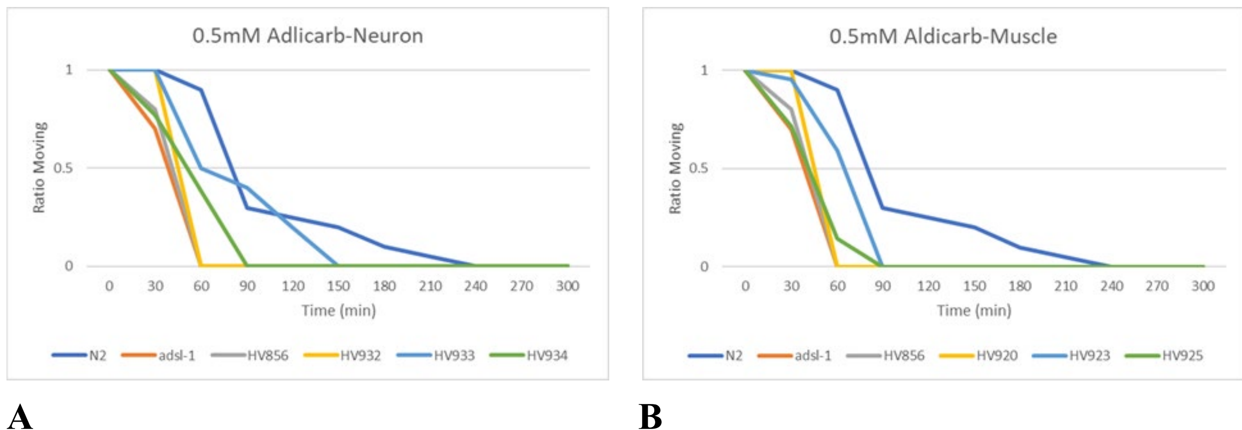

Figure 2: Ratio moving in 0.5mM Aldicarb for Transgenic *C. elegans.* All strains are exposed to aldicarb at the beginning of the fourth larval stage. (**A**) Transgenic strains with the ADSL function reinserted into the neuron compared to the control strains. (**B**) Transgenic stains with the ADSL function reinserted into the muscle compared to the control strains

Levamisole

NGM plates already spotted with OP50 E. coli were treated with levamisole diluted in 70% ethanol. The second sub hypothesis stated if ADSL is expressed only in the muscle, then the muscle function will be restored.

Then *C. elegans* were placed on the levamisole treated plates and monitored every 30 minutes. HV920 strain was the only strain to fully paralyze past the initial check (Figure 3). When levamisole was diluted the in 70% ethanol the chemical became more potent and in turn caused all the C. elegans to paralyze too quickly.

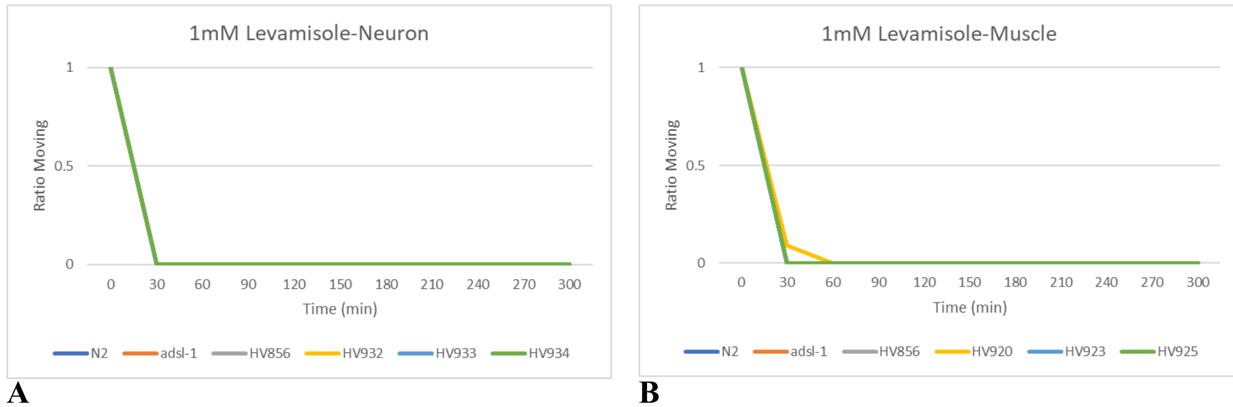

Figure 3: Ratio moving in 1mM Levamisole for Neural and Muscular Transgenic *C. elegans.* All strains are exposed to aldicarb at the beginning of the fourth larval stage. (**A**) Transgenic strains with the ADSL function reinserted into the neuron compared to the control strains. (**B**) Transgenic stains with the ADSL function reinserted into the muscle compared to the control strains

Summary

The overall hypothesis is that ADSL function is important in the muscle. The first sub-hypothesis, if ADSL is only restored in the neuron, then the muscle will remain dysfunctional is supported in Figure 2. ADSL function in the neuron reverts to normal function in Figure 2 (A) but muscle function remains dysfunctional in in Figure 2 (B). To further support the general hypothesis the second hypothesis, if ADSL is expressed only in the muscle, then the muscle function will be restored will need to reconducted. Levamisole was excessively potent so to rectify this the chemical will be diluted in water and the concentration on the plate will be reduced to 0.5mM. in doing this, I hope that there will be more of a separation between the strains so that a conclusion can be determined.

Citations

Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974;77:71–94.

Kappock, T J et al. “Modular evolution of the purine biosynthetic pathway.” Current opinion in chemical biology vol. 4,5 (2000): 567-72. doi:10.1016/s1367-5931(00)00133-2

Lewis, J A et al. “The genetics of levamisole resistance in the nematode Caenorhabditis elegans.” Genetics vol. 95,4 (1980): 905-28.

Macchiaiolo, Marina et al. “A mild form of adenylosuccinate lyase deficiency in absence of typical brain MRI features diagnosed by whole exome sequencing.” Italian journal of pediatrics vol. 43,1 65. 2 Aug. 2017, doi:10.1186/s13052-017-0383-7

Mahoney, Timothy R et al. “Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay.” Nature protocols vol. 1,4 (2006): 1772-7. doi:10.1038/nprot.2006.281

McKim, K. S., K. Peters, and A. M. Rose. "Two Types of Sites Required for Meiotic Chromosome Pairing in Caenorhabditis elegans." Genetics 134.3 (1993)749.

White, J G et al. “The structure of the nervous system of the nematode Caenorhabditis elegans.” Philosophical transactions of the Royal Society of London. Series B, Biological sciences vol. 314,1165 (1986): 1-340. doi:10.1098/rstb.1986.0056

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