Inherited Disorders of GABA Metabolism and Advances in ALDH5A1 Mutation Identification

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Abstract

Background and Objectives—Inherited disorders of GABA metabolism include SSADH and GABA-transaminase deficiencies. The clinical features, pathophysiology, diagnosis, and management of both are discussed, including an updated list of ALDH5A1 mutations causing SSADH deficiency.

Methods—Our SSADH patient database was analyzed and murine and translational studies leading to clinical trials are reviewed.

Results—The database containing 112 SSADH-deficient patients (71 pediatric and adolescent subjects, 41 adults) indicates that developmental delay and hypotonia are the most common presenting symptoms. Epilepsy is present in 2/3 of patients by adulthood. Murine genetic model, and human studies using flumazenil-PET and transcranial magnetic stimulation, have led to therapeutical trials and identified additional metabolic disruptions. Suggestions for new therapies have arisen from findings of GABAergic effects on autophagy with enhanced activation of the mTor pathway. A total of 45 pathogenic mutations have been reported in SSADH deficiency including the discovery of three previously unreported.

Conclusions—Investigations into the disorders of GABA metabolism provide fundamental insights into mechanisms underlying epilepsy and support the development of biomarkers and clinical trials. Comprehensive definition of the phenotypes of both SSADH and GABA-T deficiencies may increase our knowledge of the neurophysiological consequences of a hyperGABAergic state.

Keywords

SSADH deficiency; GABA-transaminase deficiency; neurometabolic diseases; epileptic encephalopathy; seizures

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The GABA metabolic pathway and its disruptions

After discovery by Roberts and Frankel in 1950,1 GABA was proposed to be an inhibitory neurotransmitter.2,3 GABA is in fact the brain’s major inhibitory neurotransmitter, with thirty to forty percent of cerebral synapses using it to facilitate inhibition. Only the excitatory neurotransmitter glutamate is more prevalent in the central nervous system.

GABA results from a conversion of L-glutamate via glutamate decarboxylase (GAD) (Figure). Succinic semialdehyde dehydrogenase (SSADH) and GABA-transaminase (GABA-T) work in tandem to convert GABA to succinate. Specifically, GABA-T metabolizes GABA to succinic semialdehyde, which is rapidly metabolized to succinic acid and then enters the tricarboxylic acid cycle. Through the transamination of alpha-ketoglutarate, the closed loop of this process returns to glutamate and its conversion to GABA via GAD.

For the transamination of GABA to succinic semialdehyde, alpha-ketoglutarate must be present to accept the amine group, a process which restores glutamate. The neurotransmitter pool is constantly replenished as one mole of glutamate is produced for each mole of GABA that is converted to succinate4.

In a metabolite cycle known as the glutamine-glutamate shuttle, GABA is taken up by glial cells and converted to glutamine via glutamine synthetase in the absence of GAD. Glutamine is returned to the neuron and is then converted back to glutamate via glutaminase, ultimately recycling glutamate. This completes the loop and conserves the supply of GABA precursor.

The GABA metabolic pathway is disrupted by deficiencies in GABA-T and SSADH.5,6 This review will focus on deficiencies in SSADH and GABA-T, both of which are inherited as autosomal recessive disorders.

In brief, SSADH deficiency is a rare inborn neurometabolic disorder with clinical manifestations including developmental delay and early hypotonia, later expressive language impairment and obsessive-compulsive disorder, hyporeflexia, nonprogressive ataxia and epilepsy. The diagnosis is indicated by the accumulation of GABA and its metabolite, gamma-hydroxybutyrate (GHB), in the absence of SSADH. SSADH deficiency has been identified in hundreds of patients worldwide and sequencing reveals homozygous or compound heterozygous mutations identified in the ALDH5A1 gene in nearly all families.

GABA-T deficiency is associated with neonatal seizures, profound developmental impairment, and growth acceleration. The diagnosis requires CSF analysis of GABA concentrations. GABA-T deficiency has been confirmed in two unrelated patients and suspected in an older sibling of one of these patients, who died at the age of 1 year with a clinical picture similar to that of his affected sibling.
Biochemical characteristics

The mitochondrial protein, succinate-semialdehyde dehydrogenase (SSADH) is in the aldehyde dehydrogenase family (subfamily 5A1). SSADH locates to the ALDH5A1 gene at chromosome 6p22.3 and produces succinic acid. The mitochondrial protein, 4-aminobutyrate aminotransferase (GABA-T) locates to the ABAT gene at chromosome 16p13.2. The product of GABA-T is succinic semialdehyde.

Pathophysiology of SSADH and GABA-T deficiencies

Gibson and colleagues created a mouse model of SSADH deficiency via gene targeting in which the aldha1−/− mouse developed fatal status epilepticus and died between postnatal days 20–26. The development of this murine model has facilitated numerous opportunities for researchers to reconsider the pathophysiology of general inborn metabolic disorders and SSADH deficiency in particular.

The murine model demonstrates a progression from initial absence seizures to tonic-clonic convulsions to lethal status epilepticus. Both transcranial magnetic stimulation and flumazenil-PET studies have evinced GABA-dependent down-regulation of GABA A and GABA B receptors in animal studies as well as patients. Due to the high content of the sulfonated amino acid, taurine, in dam’s milk and knowledge of lethal status epilepticus occurrence during the weaning period of newborn mice, taurine was studied and shown to significantly extend lifespan in affected mice. Also extending lifespan were the GABA B receptor antagonist CGP-35348, vigabatrin, and the high-affinity GHB receptor antagonist NCS-382, as well as a 4:1 ketogenic diet. Maximal life extension for aldha1−/− mice was provided by the GHB receptor antagonist NCS-382, suggesting for a role of GHB in the pathophysiology. Mehta and colleagues, however, did not find alterations in number of GHB receptors, binding affinity and expression in aldha1−/− brain sections. Study limitations included the possibilities that reduced lifespan prohibited GHB receptor down-regulation, the use of only a single pH for binding studies, the lack of specificity of the ligand (NCS-382), and that down-regulation of GHB receptors may have been transient. However, high-dose GHB, as found in SSADH-deficient brains, activates a specific subset of GABA B receptors, and recent studies on recombinant GABA A receptors indicate GHB activates a specific GABA A subset of receptors as well.

Other metabolites accumulate in addition to GABA and GHB, including 4,5-dihydroxyhexanoic acid and homocarnosine; furthermore, glutamine homeostasis is disrupted based on evidence from the animal model. Recent studies indicate GABA-mediated activation of the mTOR pathway, as shown by increased mitochondria in the mutant mouse, associated with increased biomarkers of oxidative stress. This suggests a potential role for the mTOR inhibitor rapamycin as well as antioxidant therapy in this disorder. Vigabatrin, an irreversible inhibitor of GABA transaminase, will reduce GHB levels as shown in CSF of treated patients. Thus, combination therapy, for example of vigabatrin and rapamycin, are in development in phase 1 animal studies.
In deficiency of the enzyme GABA-transaminase, CSF GABA levels have been reported as 16–60 times elevated. The finding of growth acceleration in GABA-T deficiency may be related to the effect of GABA on the release of growth hormone.

**METHODS**

Our SSADH patient database was analyzed and murine and translational studies leading to clinical trials are reviewed. All previously reported mutations associated with pathogenicity in SSADH deficiency were reviewed and previously unreported mutations identified within our database are presented. This study was approved by the Boston Children’s Hospital Institutional Review Board (protocol 3660).

**RESULTS**

**Clinical Characteristics**

**SSADH deficiency** is an autosomal recessive disorder identified in several hundred patients. Onset of symptoms occurs at a mean age of 11 months (range 0–44 months). The disease usually has a nonprogressive course and is difficult to distinguish from static encephalopathy, although early-onset cases may have a more severe course, with extrapyramidal signs and death in infancy, and some patients show regression.

Typical clinical manifestations are hypotonia, developmental delay, ataxia, and hyporeflexia. Nearly all patients present with hypotonia and developmental delay. The ataxia is nonprogressive and tends to improve with time. Hyporeflexia is noted on most patients on the neurological examination. Cognitive impairment is typical and expressive language deficits are prominent. In about half of patients, epilepsy usually occurs as generalized tonic-clonic seizures, although absence and myoclonic seizures occur. Sudden unexpected death in epilepsy (SUDEP) has been reported. Additionally, extrapyramidal signs may be present, including dystonia, myoclonus, and choreoathetosis.

Neuropsychiatric problems tend to be the most problematic for quality of life and are marked by inattention and aggression in early childhood, and disabling obsessive-compulsive disorder in adolescence and beyond. The most common sleep disturbance is insomnia, and overnight sleep studies show decreased stage REM percentage and prolonged latency to stage REM, with decreased mean sleep latency on daytime studies consistent with excessive daytime somnolence.

**GABA-T deficiency** has been detected in one index sibship and one additional patient from an unrelated family. Other cases may be undetected because of the general lack of testing for CSF GABA quantification. The phenotype is that of a severe neonatal or infantile epileptic encephalopathy. Clinical features reported have been neonatal seizures, lethargy, hypotonia, hyperreflexia, poor feeding, and rapid cranial growth acceleration, as well as myoclonic seizures in infancy. Neuroimaging showed severe enlargement of CSF containing spaces, and neuropathology of the index patient’s sibling showing spongiform white matter changes with decreased myelination.
Diagnosis

Diagnosis of SSADH deficiency should be taken into consideration if cases present with slowly progressive or static encephalopathy and late-infantile to early-childhood onset with the primary clinical features noted above, especially the motor and language delays.

Urine organic acid analysis, specifically gas chromatography mass spectrometry, produces the most reliable laboratory test findings. The presence of persistent GHB on urine organic acid analysis [100–1200 mmol/mol creatine (normal, 0–7 mmol/mol creatinine)] is consistent with a diagnosis of SSADH deficiency. In patients whose neuropsychiatric disturbances or developmental delays are of unknown etiology, urine organic acid analysis should be considered with specific attention to the presence of 4-OH-butyric acid.

EEG studies, while nonspecific, indicate background slowing and semirhythmic to rhythmic spike-and-wave paroxysms seen diffusely that may have asynchrony or variable hemispheric dominance. Photosensitivity and electrographic status epilepticus of slow wave sleep have been rarely recorded.

Brain MRI shows a characteristic pattern of hyperintensity of the globus pallidus, cerebellar dentate nucleus, and subthalamic nucleus. These lesions are typically, but not always, bilateral and symmetrical. Additional imaging findings are hyperintensities in subcortical white matter and brain stem, including substantia nigra, and cerebral hemispheric and cerebellar atrophy. Brain MRS shows increased endogenous GABA in brain parenchyma.

A diagnosis of SSADH deficiency may be confirmed by enzymatic quantification, although is now done by most laboratories as sequencing, or if indicated deletion/duplication testing, of the ALDH5A1 gene. Sequence analysis identifies pathogenic variants in the majority of families. Table 1 lists published mutations associated with causation of the condition as well as new mutations being reported in this manuscript.

Diagnosis of GABA-T deficiency relies on a high index of suspicion and detecting elevated amino acids in CSF, specifically GABA and beta-alanine although not GHB. MR spectroscopy for elevated GABA using voxels of the basal ganglia has been used to identify the diagnosis. Both patients confirmed were compound heterozygotes. The index patient had an A-to-G transition at a highly conserved site, affecting binding of the cofactor pyridoxal-5-phosphate, which is required for the transamination of GABA to succinic semialdehyde. The second allele was a T-to-C transition causing a substitution of leucine to proline. The unrelated patient had a missense mutation and a deletion of the GABA-transaminase coding region.

Other diagnostic issues

Prenatal diagnosis is made possible through amniocentesis for measurement of amniotic fluid GHB for SSADH deficiency, or of GABA and beta-alanine for GABA-T deficiency, or chorionic villus tissue and cultured amniocytes for metabolite measurements or gene testing. The technical feasibility of dried blood spot testing for GHB, applicable to newborn screening for SSADH deficiency, was demonstrated by Forni et al. Late diagnosis,
however, remains problematic as the non-specific clinical phenotype has caused adult patients to be improperly diagnosed or undiagnosed for decades.42

**Long-term outlook**

**SSADH deficiency** in adults causes disabling problems with obsessive-compulsive disorder and anxiety. SSADH-deficient adults in general require supervised living arrangements. Our database of 112 patients presenting with SSADH deficiency includes 41 adults age 18 years and older of which 27 (66%) have epilepsy, usually accompanied by generalized convulsive and nonconvulsive seizures.42 Reports have been made of sudden unexpected death (SUDEP),43 and progressive worsening with accelerating convulsive seizures and dementia in patients up to 63 years of age.61 Additional frequent reports of neuropsychiatric complications and sleep disturbances also persist, as well as notable anxiety and obsessive-compulsive disorder in adolescence and adulthood.

In **GABA-T deficiency**, both index family patients died at 1 and 2 years of age. The unrelated patient was alive at 28 months of age, the time of the report.

**ALDH5A1 MUTATIONS**

Pathogenic mutations in the ALDH5A1 gene (SSADH deficiency), including three previously unreported mutations from our database, are listed in the following table:

**DISCUSSION**

**Neurotoxicity and implications in development**

Although the exact mechanisms of GHB neurotoxicity in humans is still a topic of study, the neurotoxic effects of elevated endogenous GHB concentrations have shown to significantly increase various parameters of oxidative stress in recent murine studies. It is postulated that GHB neurotoxicity causes accumulation of lipid peroxidative byproducts (thiobarbituric acid reactive substances) and significantly diminishes antioxidant reactivity, thereby exacerbating neurodegeneration in patients presenting with SSADH deficiency. Excessively damaged mitochondria will induce the cell to undergo apoptosis as markedly high GHB levels in the central nervous system (CNS) likely have the capacity of causing oxidative stress. It is, however, uncertain whether this oxidative stress is a primary contributor to the onset of neurodegeneration or if its manifestation is secondary in the neurodegenerative process of SSADHD.59

It has been noted that not all SSADH-deficient patients exhibit the same response to a single potential therapeutic. The inconsistency in effectiveness of therapeutics for SSADH-deficient patients, but heterogeneity of clinical manifestations, may be due to variances in the developmental onset of circuit aberrations in SSADH deficient patients. Prior to undertaking its role as the key inhibitory neurotransmitter in mature neuronal networks, GABA is excitatory in the developing brain due to high intracellular chloride concentrations. Chronically elevated cerebral GABA and GHB concentrations during the crucial developmental stages may have different effects on neurotransmission.13
Treatment

There are no specific pharmacological strategies to address the metabolic defects. Thus, therapy is targeted, especially in terms of antiepileptic agents. Characterization of epilepsy as seen in SSADH-deficient patients guides antiepileptic drug therapy to agents used for generalized seizures. Valproate is not used first-line due to inhibition of any residual enzymatic activity, although magnesium valproate treatment controlled refractory epilepsy in a single SSADH-deficient patient, associated with improved behavior and EEG findings. Patients have shown reasonable seizure control with lamotrigine and carbamazepine. Results have been inconsistent with vigabatrin. Vigabatrin has been associated with a demonstrable decrease in CSF GHB concentration in occasional case reports. Taurine was associated with developmental improvement in a two-year-old boy in an anecdotal report, while an open label clinical trial did not yield significant improvements in adaptive behavioral functioning.

Novel directions

Studies of SSADH and reports of GABA-T deficiencies have engendered new discernments into GABA-related biology, expanding our understanding of GABA beyond its role as an inhibitory neurotransmitter. They also provide opportunities to rethink fundamental questions encompassing epileptic mechanisms. The rarity of GABA-transaminase deficiency suggests the mutation may be lethal in utero or that the phenotype is far from defined, with lack of CSF GABA determination being a major diagnostic obstacle.

The SSADH murine knockout model has informed us of the neurophysiological consequences of a hyperGABAergic state, accumulation of GABA, GHB, and related metabolites and disruption of the glial-neuronal glutamine shuttle. Overuse dependent downregulation of both GABA(A) and (B) receptors has been demonstrated in the animal model, and then replicated in affected patients whereas flumazenil-PET showed decreased GABA(A) receptor binding, and transcranial magnetic stimulation (TMS) indicated reduction of the cortical silent period and long interval intracortical inhibition consistent with GABA(B) receptor underactivity. This work has led to clinical trials, from taurine intervention to a currently enrolling trial of the GABA_B receptor antagonist, SGS-742 (www.clinicaltrials.gov; NCT01608178; NCT02019667). Implementation of a combination of interventions may be necessary to address the complex clinical representation and offer maximum benefit.

Acknowledgements

Thank you to Shaye Moore and Elizabeth Jarvis for their technical assistance. Grant/research support from NIH/NINDS: NCT02019667.

REFERENCES


Dev Med Child Neurol. Author manuscript; available in PMC 2016 June 29.


What this paper adds

1. Translational studies from a murine knockout model confirm overuse dependent downregulation of GABA\textsubscript{A} and GABA\textsubscript{B} receptors in patients.

2. Biomarker development during translational trials has enabled a pathway to clinical trials now in progress for subjects with SSADH deficiency.

3. An updated comprehensive table of published mutations identified in ALDH5A1 including three previously unreported.
**Figure.**
The metabolic pathway of gamma-aminobutyric acid (GABA) and potential targets for therapeutic trials in SSADH deficiency.

- **GAD** = glutamic acid decarboxylase
- **P5P** = pyridoxal-5'-phosphate
- **VGB** = vigabatrin
- **GABA-T** = GABA-transaminase
- **SSADH** = succinic semialdehyde dehydrogenase
- **GABABR** = GABA subunit B receptor
- **GABAAR** = GABA subunit A receptor
- **mTOR** = mammalian target of rapamycin
- **SGS742**
- **Rapamycin**
- **Antioxidants**
- **Mitophagy**
- **Pexophagy**
## Table

Summary of Disease-Associated Mutations in ALDH5A1 (SSADH Deficiency)

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(* previously unpublished)