Inherited disorders of GABA metabolism

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The inherited disorders of γ-amino butyric acid (GABA) metabolism require an increased index of clinical suspicion. The known genetic disorders are GABA-transaminase deficiency, succinic semialdehyde dehydrogenase (SSADH) deficiency and homocarnosinosis. A recent link has also been made between impaired GABA synthesis and nonsyndromic cleft lip, with or without cleft palate. SSADH deficiency is the most commonly occurring of the inherited disorders of neurotransmitters. The disorder has a nonspecific phenotype with myriad neurological and psychiatric manifestations, and usually has a nonprogressive temporal course. Diagnosis is made by the detection of γ-hydroxybutyrate excretion on urine organic acid testing. The most consistent magnetic resonance imaging abnormality is an increased signal in the globus pallidus. Magnetic resonance spectroscopy has demonstrated the first example of increased endogenous GABA in human brain parenchyma in this disorder. GABA-transaminase deficiency and homocarnosinosis appear to be very rare, but require cerebrospinal fluid for detection, thus allowing for the possibility that these entities, as in the other inherited neurotransmitter disorders, are under-recognized.

Keywords: γ-amino butyric acid, γ-hydroxybutyrate, homocarnosine, seizures, succinic semialdehyde dehydrogenase, vigabatrin

future medicine

Up to a third of cerebral synapses employ γ-amino butyric acid (GABA), the major inhibitory neurotransmitter of the brain. Its major precursor is L-glutamate, which is converted to GABA via the enzyme glutamate decarboxylase (GAD). GAD has two active isoforms, GAD65 and GAD67. Pyridoxal-5-phosphate is a coenzyme for GAD. GABA is metabolized by the enzyme GABA-transaminase (GABA-T), to succinic semialdehyde. This unstable intermediate compound is metabolized rapidly to succinic acid, which enters the tricarboxylic acid cycle. The so-called GABA shunt is a closed loop that involves the transamination of α-ketoglutarate to glutamate, which is then converted via GAD to GABA (**Figure 1**). The subsequent transamination of GABA to succinic semialdehyde requires the presence of α-ketoglutarate to accept the amine group. Thus, this restores glutamate and a molecule of the GABA precursor is formed as a molecule of GABA is catabolized. This enables constant replenishment of this vital neurotransmitter pool. There is an ancillary loop, known as the glutamine–glutamate shuttle. Released GABA is taken up by glial cells, where glutamate can be formed, but not converted to GABA, owing to an absence of GAD. Instead, GABA is converted via glutamine synthetase to glutamine, which is returned to the neuron and is converted via glutaminase back to glutamate. Thus, the loop is completed and the supply of GABA precursor is conserved.

Disorders involving the GABA catabolic pathway are GABA-T deficiency, succinic semialdehyde dehydrogenase (SSADH) deficiency and homocarnosinosis; all of these entities invoke neurological dysfunction. SSADH deficiency is the most common, but has a heterogeneous, nonspecific phenotype. Enzymatic deficiency can be documented in SSADH and GABA-T deficiency. Homocarnosine is a dipeptide compound consisting of GABA and histidine.

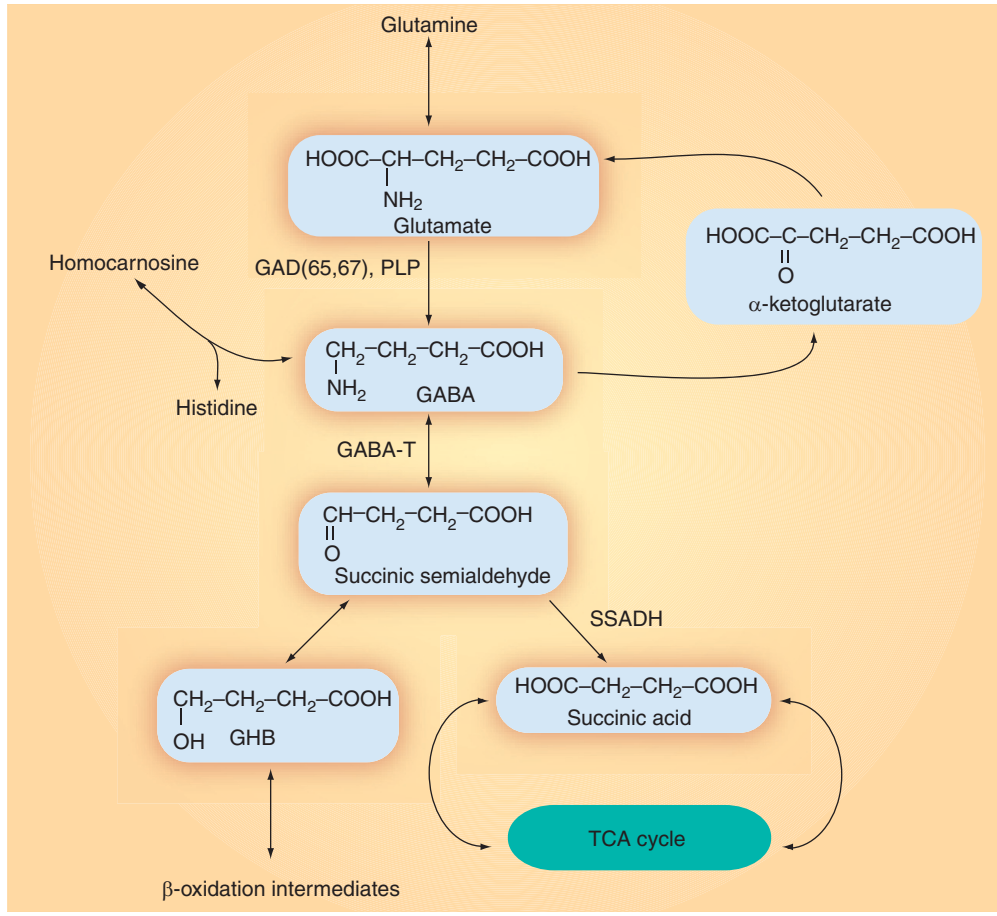
Inherited disorders of GABA synthesis are less well defined. Linkage between GAD67 deficiency and nonsyndromic cleft lip and palate has been demonstrated. While pyridoxine-dependent epilepsy has traditionally been cast as a disorder of GABA synthesis, no metabolic or genetic aberration of GABA production has been documented in this group of disorders. Furthermore, recent data confirm abnormalities of different metabolic pathways, including deficiency of α-amino-adipic dehydrogenase leading to the accumulation of pipecolic acid and sequestration of pyridoxine, in this disorder. This topic is therefore addressed in a separate article in this issue of *Future Neurology*.

Inherited disorders of GABA metabolism

GABA-transaminase deficiency

GABA-T deficiency is an autosomal recessive disorder characterized by abnormal development, seizures and high levels of GABA in serum and cerebrospinal fluid (CSF) [1]. The disorder appears to be extremely rare. It was reported

Figure 1. γ-amino butyric acid metabolic pathway.



initially in two of four siblings in a single Flemish family. Since then, another, unrelated patient has been reported, but enzyme activity for this patient was within normal limits in another laboratory, and disease-associated mutations were not identified.

The clinical manifestations noted in the two affected siblings included intractable neonatal seizures (clonic and tonic), lethargy, hypotonia, hyper-reflexia, poor feeding, severely retarded psychomotor development and a high-pitched cry. Linear growth was accelerated, with a notable rapid increase in the period immediately preceding the male sibling's death. The linear growth is attributable to a positive effect of GABA on growth hormone production. The female sibling initially had a normal electroencephalogram (EEG) at 2 weeks of age, but later developed low-voltage β-frequency activity with intermittent epileptiform discharges, and, at 2 years of age, demonstrated generalized epileptiform paroxysms. Computed tomography (CT) in both siblings revealed severe ventricular enlargement in addition to increased cisternal and sulcal spaces. Both patients succumbed to the disorder, with the male dying at 1 and the female at 2 years of age. Post-mortem examination in one showed spongiform leukodystrophy.

Determination of CSF amino acids demonstrating significantly elevated levels of GABA (total and free) and β-alanine levels, provides the diagnosis of GABA-T deficiency. Plasma levels of these metabolites are also elevated, although not as significantly. Aminoaciduria is also present. Two different missense mutations were identified in the family reported [2]. A definitive diagnosis can be made by the measurement of GABA-T activity in the liver, lymphocytes isolated from whole blood or Epstein–Barr virus-transformed cultured lymphocytes.

Succinic semialdehyde dehydrogenase deficiency

SSADH deficiency is autosomal recessive and has been identified in approximately 400 people worldwide. Owing to the enzyme deficiency,

Table 1. Clinical features in SSADH deficiency (n = 53).

Clinical findings	***(n = 53)***
Developmental delay	53 (100%)
Behavior problems	37 (70%)
Mental retardation	43 (81%)
Hypotonia	35 (66%)
Ataxia	28 (53%)
Seizures	24 (45%)
Neuropsychiatric problems	***(n = 37; 70%)***
Sleep disturbance	25 (68%)
Inattention	19 (51%)
Hyperactivity	15 (41%)
OCD	13 (35%)
Anxiety	11 (30%)
Aggression	6 (16%)
Hallucinations	4 (11%)
PDD/autism	1 (3%)
EEG studies	***(n = 28; 53%)***
Spike discharges	8 (29%)
Background abnormal/slowing	7 (25%)
Photosensitivity	2 (7%)
ESES	1 (4%)
Normal	10 (36%)
Seizures	***(n = 25; 47%)***
Generalized tonic–clonic	14 (56%)
Myoclonic	5 (20%)
Absence	10 (40%)
Other (ALTE, atonic, partial and febrile)	7 (28%)
Unspecified	3 (12%)
Neuroimaging	***(n = 30; 57%)***
Increased T2 signal:	
Globus pallidi	13 (43%)
Dentate nucleus	5 (17%)
White matter	2 (7%)
Brainstem	2 (7%)
Normal MRI	13 (43%)
Cerebral atrophy	3 (10%)
Cerebellar atrophy	2 (7%)
Delayed myelination	2 (7%)

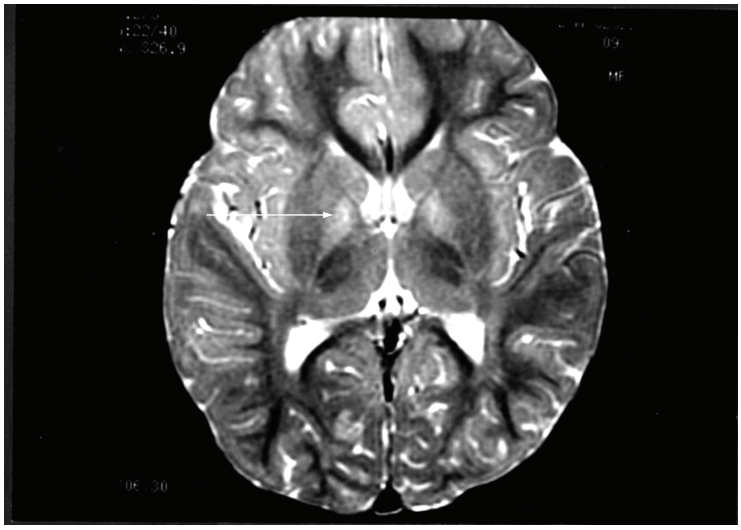
Based on systematic questionnaire study. Questionnaire approved by the Childrens National Medical Center Institutional Review Board.
ALTE: Apparent life-threatening event; EEG: Electroencephalogram; ESES: Electrical status eplilepticus in sleep; MRI: Magnetic resonance imaging; OCD: Obsessive compulsive disorder; PDD: Pervasive developmental disorders; SSADH: Succinic semialdehyde dehydrogenase.
Reprinted with permission from [8] © 2005 Canadian Society of Clinical Chemists.

GABA is not converted to succinic acid, but accumulates as γ-hydroxybutyrate (GHB) (**Figure 1**). It is unclear whether elevated GABA, GHB or other neurometabolic changes account for the phenotype; however, it is known that the primary metabolic abnormality is an excessive concentration of GHB in physiological fluids, with elevations up to 800-fold in urine and 200-fold in plasma. Elevations in CSF of GHB are 65- to 230-fold, and in total GABA, up to threefold [3]. The gene has been mapped to chromosome locus 6p22. More than 35 mutations have been identified, including missense, nonsense and splicing errors. No hotspots were detected [4].

The index case was reported by Jakobs in 1981 [5]. Sporadic case reports appeared subsequently, with series of patients subsequently reported to clarify the clinical spectrum of the disorder [6,7]. This disorder presents in childhood with nonspecific features, thus developmental delay and subsequent mental retardation with predominantly expressive language impairment, in addition to hypotonia, mild ataxia and hyporeflexia (**Table 1**) [8]. Seizures occur in approximately half of all patients. Generalized seizures are the most prevalent, particularly tonic–clonic, although absence and myoclonic seizures occur. More than two-thirds of SSADH patients suffer from neuropsychiatric problems, particularly nonspecific sleep disturbances, inattention, hyperactivity, obsessive–compulsive disorder and anxiety. While the disorder does not generally manifest a degenerative course or intermittent decompensation, episodes of unexplained lethargy and developmental regression have occurred in occasional patients, and there appears to be a more fulminant course in some early-onset patients, with seizures, choreoathetosis, myoclonus, optic atrophy, dystonia and death in infancy [9].

Imaging studies have demonstrated increased T2-weighted signal in the globus pallidi (**Figure 2**). Other abnormalities include cerebral and cerebellar atrophy, T2-weighted hyperintensities in subcortical white matter, cerebellar dentate nucleus, brainstem and delayed myelination. Magnetic resoncance imaging (MRI) was read as normal for 43% of the affected individuals [8]. Positron emission tomography studies utilizing fluorodeoxyglucose have shown cerebellar hypometabolism in two patients with known cerebellar atrophy and normal findings in one other patient [7]. While 3H-MRI spectroscopy for standard neuronal

Figure 2. MRI in succinic semialdehyde dehydrogenase deficiency.



The arrow indicates bright globus pallidus on T2-weighted image.

markers, such as *N*-acetylasparatate, choline and creatine, have been normal, with no identification of a lactate peak, specialized magnetic resonance scanning (MRS) edited for neurotransmitters has revealed elevations of GABA in occipital lobe parenchyma [10,11].

Diagnosis is made initially by identifying urinary excretion of 4-hydroxybutyric acid measured by specific ion monitoring on gas chromatography (GC)/mass spectrometry (MS). Routine organic acid analysis may not be a sufficient method of detection and selective ion monitoring, in addition to total ion chromatography, is helpful to detect GHB [7].

There is currently no standard treatment for individuals with SSADH deficiency. However, several therapies have been pursued, mainly with the intent of symptomatic treatment. Vigabatrin, an irreversible inhibitor of GABA-T, has been associated with decreases in CSF GHB. While there has been an interest in following CSF GHB levels during therapy with vigabatrin in patients with SSADH deficiency [12], neither laboratory or clinical effects have been consistent with vigabatrin therapy [13]. Benzodiazepines, risperidal, fluoxetine and methylphenidate have been useful therapeutics for anxiety and behavioral problems [3]. Symptomatic treatment for seizures using carbamazepine and lamotrigine have also yielded some success. Valproate is avoided, as it inhibits the activity of residual SSADH, and its use is associated with an increased concentration of GHB and other SSADH-deficiency metabolites [14].

Homocarnosinosis

Homocarnosine is a brain-specific dipeptide of GABA and histidine. Its synthesis, as with carnosine, is via carnosine synthetase and its catabolism is via serum carnosinase. Homocarnosine concentrations are highest in the dentate and inferior olivary nuclei, intermediate in substantia nigra and globus pallidus, and lowest in frontal cortex, caudate nucleus and nucleus accumbens [1]. One affected family has been reported with homocarnosinosis, affecting a healthy 72-year-old Norwegian woman and three of her four children with neurological disease. Their phenotype was onset of progressive spastic paraplegia between 6 and 29 years of age with progressive mental deterioration and retinal pigmentation. The patients had elevated CSF homocarnosine and normal carnosine. The disorder is thought to likely represent a form of carnosinase deficiency.

Nonsyndromic cleft lip with or without cleft palate

GABA has an important role in embryonic development, especially facial development, as substantiated by the association of cleft palate in transgenic mice deficient in the GAD67 [15]. A recent Japanese population study reported linkage in patients with nonsyndromic cleft lip with or without cleft palate and specific GAD67 haplotypes [16].

Conclusion

The inherited disorders of GABA metabolism are GABA-T deficiency, SSADH deficiency, homocarnosinosis and nonsyndromic cleft lip with or without cleft palate. Each invokes important roles for GABA in embryogenesis and brain development. Pyridoxine dependency was traditionally considered a disorder of GABA synthesis manifest by dramatic epileptogenicity. However, more recent data have not supported this concept and has invoked other metabolic aberrations. SSADH deficiency is the most common of the inherited neurotransmitter disorders and is diagnosed by urine organic acid analysis for the presence of GHB, with subsequent confirmation by enzymatic assay available in leukocytes. The phenotype is nonspecific and includes developmental delay, cognitive retardation predominantly affecting expressive language, hypotonia, nonprogressive cerebellar ataxia, hyporeflexia, behavioral abnormalities and seizures. Neuroimaging most frequently shows pallidal hyperintensity

or a dentatopallidal pattern. The other GABA catabolic disorders are exceedingly rare and require CSF study for detection. In general, there is much to be learned about this intriguing group of disorders and an increased index of clinical suspicion is required to identify patients.

Future perspective

While rare, the genetic disorders of GABA metabolism constitute the most common of the inherited disorders of neurotransmitters. In addition, they pose research questions regarding a range of highly prevalent and significant disorders in neurobiology. SSADH deficiency alone encompasses the problems of mental retardation, epilepsy, sleep disorders and severe psychiatric symptomatology. The endogenous elevation of GHB, a substance with known abuse potential, is relevant to the problem of drug addiction. More recent investigations in SSADH deficiency invoke a role for $GABA_A$ receptor-mediated epileptogenesis [17], downregulation for genes associated with myelin biogenesis in gene-expression profiling technology [18] and refined stable-isotope dilution liquid chromatography-tandem MS methodology for the determination of succinic semialdehyde [19]. These innovative experimental studies approach fundamental questions in the areas of epileptogenesis, myelination and laboratory technology, respectively. In addition, liver-mediated gene therapy in the murine SSADH model has shown a reduction in GHB levels in the liver, kidney, serum and brain extracts, setting the stage for future clinical trials of gene therapy [20].

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Executive summary

Introduction

- Inherited disorders involving the γ-amino butyrate acid (GABA) catabolic pathway are GABA-transaminase (GABA-T) deficiency, succinic semialdehyde dehydrogenase (SSADH) deficiency and homocarnosinosis.
- All of these invoke neurological dysfunction. SSADH deficiency is the most common, but has a heterogeneous, nonspecific phenotype.
- Nonsyndromic cleft lip with or without cleft palate has been linked to inherited haplotypes leading to GAD67 deficiency, a disorder of GABA synthesis.

Inherited disorders of GABA metabolism

- The biochemical hallmark of SSADH deficiency is the accumulation of γ-hydroxybutyrate (GHB) in physiologic fluids, including blood, urine and cerebrospinal fluid. Elevation has also been documented in brain parenchyma, via magnetic resonance (MR) spectroscopy.
- SSADH deficiency presents with a constellation of neuropsychiatric signs, including developmental delay, mental retardation particularly affecting expressive language, hypotonia, cerebellar ataxia, hyporeflexia, sleep disturbances, aggression, obsessive–compulsive disorder, inattention and hyperactivity. Epilepsy affects approximately half of patients and is usually generalized.
- Neuroimaging in SSADH deficiency reveals increased T2-weighted signal in the globus pallidi or a dentatopallidal pattern. Abnormal signaling has also been noted in subcortical white matter and the brainstem.
- Generalized convulsions are a prominent finding in some of these patients with this hyperGABAergic condition, leading to a requriment for new hypotheses on the role of GABA in convulsive seizures.
- There is no standard therapy for SSADH deficiency; vigabatrin, while a logical choice to avoid γ-hydroxybutyrate production, has not been consistently useful.
- Cerebrospinal fluid analysis is needed to diagnose GABA-T deficiency and homocarnosinosis.

Conclusions

- SSADH deficiency usually presents with a nonspecific encephalopathy with a static tempo, although 10% of patients have a more severe course, featuring extrapyramidal involvement and a progressive course.
- MR imaging reveals abnormalities suggestive of an organic acidopathy, and the detection of GHB on urine organic acid analysis leads to the diagnosis.

Future research work

- The murine model has led to innovative findings with potential applicability of high significance to human conditions, including a role for $GABA_A$ receptor dysfunction or downregulation in the genesis of generalized epilepsy, and preliminary success in achieving desirable metabolic outcomes utilizing gene therapy.

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