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REVIEW

Therapeutic concepts in succinate semialdehyde dehydrogenase (SSADH; ALDH5a1) deficiency (γ-hydroxybutyric aciduria). Hypotheses evolved from 25 years of patient evaluation, studies in *Aldh5a1*$^{-/-}$ mice and characterization of γ-hydroxybutyric acid pharmacology

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Summary We overview the pathophysiological bases, clinical approaches and potential therapeutic options for succinate semialdehyde dehydrogenase (SSADH; EC1.2.1.24) deficiency (γ-hydroxybutyric aciduria, OMIM 271980, 610045) in relation to studies on SSADH gene-deleted mice, outcome data developed from 25 years of patient evaluation, and characterization of γ-hydroxybutyric acid (GHB) pharmacology in different species. The clinical picture of this disorder encompasses a wide spectrum of neurological and psychiatric dysfunction, such as psychomotor retardation, delayed speech development, epileptic seizures and behavioural disturbances, emphasizing the multifactorial pathophysiology of SSADH deficiency. The murine SSADH$^{-/-}$ (e.g. *Aldh5a1*$^{-/-}$) mouse model suffers from epileptic seizures and succumbs to early lethality. *Aldh5a1*$^{-/-}$

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mice accumulate GHB and γ-aminobutyric acid (GABA) in the central nervous system, exhibit alterations of amino acids such as glutamine (Gln), alanine (Ala) and arginine (Arg), and manifest disturbances in other systems including dopamine, neurosteroids and antioxidant status. Therapeutic concepts in patients with SSADH deficiency and preclinical therapeutic experiments are discussed in light of data collected from research in *Aldh5a1*$^{-/-}$ mice and animal studies of GHB pharmacology; these studies are the foundation for novel working approaches, including pharmacological and dietary trials, which are presented for future evaluation in this disease.

Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
ALLO	allopregnanolone; 3α-hydroxy-5α-tetrahydroprogesterone
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
BBB	blood–brain barrier
BHB	β-hydroxybutyrate
D-2-HG	D-2-hydroxyglutarate
DHA	docosahexaenoic acid
DHHA	dihydroxyhexanoic acid
GABA	γ-aminobutyric acid
GABA(B)R	GABA(B) receptor
GABA-T	GABA transaminase
GBL	γ-butyrolactone
GHB	γ-hydroxybutyric acid
GHBR	GHB receptor
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA synthase
HOT	hydroxyacid–oxoacid transhydrogenase
HVA	homovanillic avid
MAO	monoamine oxidase
MAP	mitogen-activated protein
NMDA	*N*-methyl-D-aspartate
PPAR	peroxisome proliferator-activated receptor
SSA	succinic semialdehyde
SSADH	succinate semialdehyde dehydrogenase
T-HCA	*trans*-4-hydroxycrotonic acid

Introduction

SSADH (EC 1.2.1.24) deficiency (γ-hydroxybutyric aciduria; OMIM 271980, 610045) is an autosomal-recessively inherited neurometabolic disorder of GABA metabolism impairing the major oxidative conversion of succinic semialdehyde (SSA) to succinic acid (Fig. 1). The genetic block leads to accumulation of SSA, which is converted to GHB. Patients present to a variable extent with retardation of speech, intellectual and also motor development.

The genetic basis resides in the SSADH gene (i.e. aldehyde dehydrogenase 5 family, member A1: ALDH5A1), which maps to chromosome 6p22. Jakobs and co-workers described the first patient with γ-hydroxybutyric aciduria, who was identified by striking accumulation of GHB in body fluids (Jakobs et al 1981). Enzyme deficiency was demonstrated in blood lymphocytes (Gibson et al 1983, 1985, 1991). Since the first description, numerous patients have been identified (Pearl et al 2003).

To examine pathophysiological mechanisms and pharmacotherapeutic approaches, Hogema and co-workers developed a mouse model of *Aldh5a1*$^{-/-}$ mice (Hogema et al 2001). *Aldh5a1*$^{-/-}$ mice progressively undergo generalized absence seizures starting in the neonatal period, followed by tonic-clonic status epilepticus by the 3rd to 4th week of life and, eventually, early death (Gupta et al 2004). *Aldh5a1*$^{-/-}$ mice are characterized by disturbances of neural transmission and amino acid homeostasis along with imbalance in excitatory/inhibitory signals. These imbalances predominantly involve GHB and GABA, but also glutamine (Gln), arginine (Arg), dopamine and other central-acting systems (Jansen et al 2006). GHB exhibits physiological and neuropharmacological properties acting on high- and low-affinity GHB receptors (GHBR) in the CNS and, at higher concentrations, on the GABA(B) receptor (GABA(B)R) (Gibson et al 2002; Lingenhoehl et al 1999). GABA predominantly exerts an inhibitory role in the brain but functions dually in the developing neuronal network where it provides trophic functions and excitatory input for immature neurons (Kirmse and Kirischuk 2006). In SSADH deficiency other transmitter systems also appear disturbed, such as glutamate (Glu), which is derived from Gln using an intercellular shunt from glial cells to neurons and serves as the major excitatory transmitter in the CNS (Lebon et al 2002).

Pharmacologically, GHB readily passes the blood–brain barrier (BBB) to enter the CNS after exogenous administration; it represents a mood-affecting sedative drug and also a treatment option, e.g. for alcohol addiction (Scharf et al 1985; Shannon and Quang 2000) and cataplexy (Fuller et al 2004). Chronic administration of GHB, or its precursor γ-butyrolactone (GBL), produces physical dependence along with activation of the GABA(B)R and withdrawal phenomena, as shown in baboons (Goodwin et al 2006; Weerts et al 2005). Acutely intoxicated patients may present with sedation, amnesia, coma or even death, but may also show

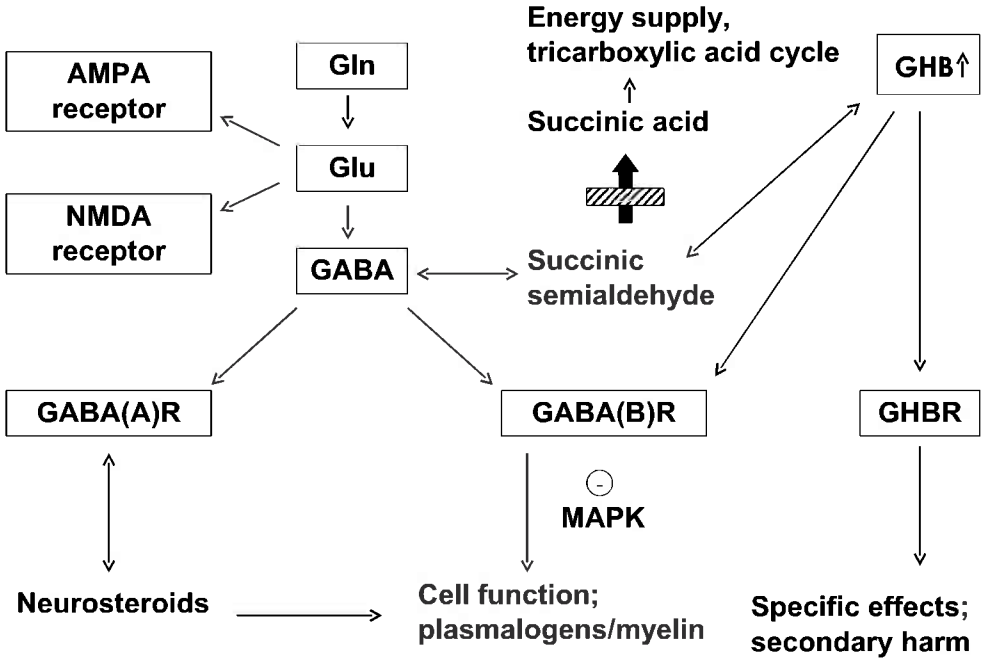

Fig. 1 Putative pathophysiological interrelationships in SSADH deficiency. The enzyme defect is marked by the hatched arrow. Pathophysiologically, GABA and GHB downregulate GABA receptors. GABA, acting via the GABA(A)R, may result in decreased production of neuroactive steroids by lowering the activity of 3-β-hydroxysteroid dehydrogenase activity; alterations in neurosteroid levels are likely to have an allosteric effect on the GABA(A)R. GHB, acting via both GHB and GABA(B)R activation, reduces signalling via a decrease in mitogen-activated protein kinase phosphorylation; MAPK is important in myelin basic protein (MBP) expression, a major component of myelin. Neurosteroids such as allopregnanolone are able to regulate myelination in a concerted manner through steroid receptors and also through GABA(A)R (Donarum et al 2006)

paradoxical agitation; as an illicit drug, GHB is also used for drug-facilitated sexual assault ('date rape') owing to its rapid action (Drasbek et al 2006). We discuss empirical and theoretical therapeutic approaches based on clinical experiences gleaned from over two decades of patient evaluation, studies of *Aldh5a1*$^{-/-}$ mice and more than 40 years of evaluation of GHB pharmacology in different species (Wong et al 2004).

Clinical phenotype of human SSADH deficiency

SSADH deficiency is a disorder that manifests predominantly with neurological findings but has considerable phenotypic heterogeneity (Gibson et al 1997; Pearl et al 2003). The clinical picture encompasses a wide spectrum of neurological manifestations and psychiatric dysfunction, and predominantly leads to a neurodevelopmental disorder with language deficits. The patients usually present with mild to severe developmental delay, predominantly involving expressive language. Other typical clinical signs and symptoms include hypotonia, truncal or appendicular ataxia, and hyporeflexia. Patients often develop neuropsychiatric symptoms such as inattention, anxiety, hyperkinesis, sleep disturbances and excessive daytime somnolence (Gibson et al 1997, 2003; Pearl et al 2003, 2005a,b; Philippe et al 2004).

In contrast to the animal model, the clinical course in patients may be static, typically with improvement in gait ataxia over time. In contrast, there is a minority of patients (approximately 10%) with a progressive course featuring developmental regression and substantial extrapyramidal manifestations, including dystonia, choreoathetosis and myoclonus (Pearl et al 2005a). A larger series of older patients indicates that ataxia and language disabilities may show improvement with age; however, language dysfunction and psychiatric symptoms remain the predominant handicaps in adult patients (Pearl et al 2005a).

About half of the patients with SSADH deficiency suffer from epilepsy, usually absence, myoclonic epilepsy or generalized tonic-clonic seizures (Pearl et al 2005b). In contrast to the *Aldh5a1*$^{-/-}$ mice, epileptic seizures only occasionally progress to refractory epilepsy or generalized convulsive status epilepticus.

Electroencephalographic (EEG) recordings in affected patients may exhibit background slowing and disorganization and epileptiform discharges (Pearl et al 2006). The latter are usually generalized spike-and-wave between 2 and 3 Hz; photosensitivity and electrographic status epilepticus of slow-wave sleep have been noted rarely. Neuroimaging with magnetic resonance techniques (MRI) reveals cerebral and cerebellar (especially vermian) atrophy and enhanced T2-weighted signal intensities involving the globus pallidus, subcortical white matter, dentate nucleus and brainstem including substantia nigra (Pearl and Gibson 2004). By means of ^{3}H MRI spectroscopy (MRS) up to threefold elevation of GABA concentrations have been described in the brain parenchyma of SSADH-deficient patients (Novotny et al 2003). Positron emission tomography (PET) using fluorodeoxyglucose has demonstrated cerebellar hypometabolism in patients with known cerebellar atrophy, without other parenchymal findings (Pearl et al 2003). SSADH deficiency has rarely been detected in association with a second genetic disorder, e.g. in a patient with WAGRO syndrome (Wilms' tumour, aniridia, genital abnormalities, mental retardation, and obesity) (Jung et al 2006) and Williams–Beuren syndrome (Knerr et al 2007).

Metabolic phenotype

In contrast to other inborn errors of metabolism such as the 'classical' organic acidopathies, patients with SSADH deficiency, while presenting with considerable accumulation of GHB in biological fluids, do not present with metabolic acidosis, hyperammonaemia or hypoglycaemia but may have significant medium-chain dicarboxylic aciduria (Gibson et al 1989b). Carnitine status should be tested during metabolic work-up, although there are only a few cases presenting with low carnitine concentrations and muscular hypotonia (Gibson et al 1997). Interestingly, in *Aldh5a1*$^{-/-}$ mice, free carnitine and acylcarnitine concentrations in serum, quantified by tandem mass spectrometry (MS/MS), do not exhibit any alteration compared to wild-type mice and there is no difference in C_4-OH carnitine levels, the expected GHB carnitine ester, between genotypes (Struys et al 2006b).

Concentrations of GHB can be routinely determined by combined gas chromatography and mass spectrometry (GC-MS), optimally using a quantitative stable-isotope dilution assay (Gibson et al 1990). However, accuracy of detection varies considerably between different laboratories (Bonham et al 1994). Variable excretion of GHB in urine may hamper detection using routine organic acid analyses. A diagnostic pitfall is the application of GHB as sedative, e.g. prior to invasive examination of developmental delay in young children (Wolf et al 2004).

Quantitation of urinary GHB excretion for the clinical course is not useful for prognostic purposes. Other compounds may be detectable in SSADH deficiency, owing to alternative metabolism, such as 3,4-dihydroxybutyric acid, 3-oxo-4-hydroxybutyric acid, glycolic acid, 2,4-dihydroxybutyric acid and, to a lesser extent, *threo*- and *erythro*-4,5-dihydroxyhexanoic acids and their lactones, the latter quite specific for this disorder. None the less, the white blood cell enzyme assay remains the gold standard for diagnostic confirmation (Gibson et al 1983, 1985, 1991). The frequently observed dicarboxylic aciduria in patients with SSADH deficiency may suggest a secondary inhibition of mitochondrial fatty acid β-oxidation, but there is evidence against this theory (Gibson and Nyhan 1989). Propionyl-CoA metabolism may also be affected as shown by an enhanced excretion of 3-hydroxypropionic acid in patients with SSADH deficiency (Brown et al 1987).

CSF GHB is up to 230-fold elevated in patients, whereas GABA is up to three times higher and Gln is decreased, suggestive of a disruption of the Gln/Glu/GABA shuttle between glial cells and neurons (Gibson et al 2003). Interestingly, homovanillic avid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) appear to exhibit a positive correlation with GHB concentration, perhaps suggesting enhanced dopamine and serotonin turnover and progressive deterioration of neuronal function (Gibson et al 2003). However, this observation requires further evaluation with respect to patient age.

Enzyme phenotype and superfamily

$NAD^{(+)}$-dependent SSADH is a mitochondrial matrix enzyme that has high specificity for SSA. SSADH has been purified from rat and human brain and liver, respectively, and its activity can also be determined in peripheral lymphocytes and cultured lymphoblasts using radiometric or spectrofluorometric assays (Chambliss et al 1995; Gibson et al 1985, 1991; Nguyen and Picklo 2003). Prenatal diagnosis has been successfully performed using isotope-dilution MS to assess quantities of GHB in amniotic fluid, determination of SSADH activity in chorionic villus samples, and molecular genetic analysis (Akaboshi et al 2003; Gibson et al 1990). SSADH activity may be a target

of inactivation in instances of elevated oxidative stress and lipid peroxidation (Nguyen and Picklo 2003).

Other enzymes are involved in the metabolism of SSA. The aldo-keto reductases, in particular, make up a superfamily of enzymes that can reduce a variety of aldehydes and ketones to their corresponding alcohols. Each family displays distinct preferences for certain substrates, presumably reflecting their role within the cell. The AKR7A subfamily shows higher affinities for SSA than does AKR7A1 (Zhu et al 2006). The SSA reductase (SSAR) is also a member of the aldo-keto reductase family 7A2 (AKR7A2; Hinshelwood et al 2002, 2003). In contrast to the mitochondrial localization of SSADH, SSAR is located within the cytoplasm. Additionally, a mammalian hydroxyacid–oxoacid transhydrogenase (HOT) which was recently cloned and resides on human chromosome 8q 13.1, catalyses the α-ketoglutarate-dependent oxidation of GHB to SSA (Kaufman and Nelson 1991; Kaufman et al 1988; Kardon et al 2006). HOT could be a therapeutic target in SSADH deficiency. Unfortunately, despite the capacity to reduce GHB levels, HOT stoichiometrically generates a potentially neuroactive by-product, D-2-hydroxyglutarate (D-2-HG; Struys et al 2005, 2006a). Elevated D-2-HG is found in physiological fluids derived from patients with SSADH deficiency (Struys et al 2005, 2006b), and baboons produce D-2-HG following administration of GHB (Struys et al 2006a). These data provide evidence that HOT is active in a number of mammalian species.

Molecular genotype

The human *ALDH5A1* gene maps to chromosome 6p22 and consists of 10 exons covering 38 kb of DNA (Chambliss et al 1998). Over 40 mutations have been identified thus far including missense, nonsense and splicing errors; however, no hotspots have been identified (Akaboshi et al 2003). Consanguinity is frequent, suggesting the occurrence of rare disease-causing alleles in the general population. There is no apparent correlation between phenotype and underlying genotype, and, additionally, most mutations reported are private. Heterozygote carriers are apparently asymptomatic, yet one report suggests absence epilepsy could be associated with the heterozygous state (Dervent et al 2004). In *Aldh5a1*$^{-/-}$ mice the heterozygous animals are apparently normal (Gibson et al 2002, 2005). Comparative studies on polymorphisms between humans and baboons suggest a putative role for the SSADH gene in the evolution of cognitive capabilities unique to humans (Blasi et al 2006).

Pathophysiological aspects: effects of GHB, GABA, and other metabolites

Deficiency of SSADH is associated with a significant elevation of GHB and total GABA in knockout mice, similar to alterations detected in patients (Gibson et al 2002; Hogema et al 2001). At physiological concentrations, GHB primarily acts at the GHBR, which is located presynaptically and functions as a G protein-coupled receptor. The pharmacological actions of high GHB concentrations are likely mediated through activation of the GABA(B)R. GHB has no affinity for the GABA(A) receptor (GABA(A)R; Castelli et al 2003; Mathivet et al 1997). In *Aldh5a1*$^{-/-}$ mice, increased levels of GABA and GHB alter GABA(B)R and GABA(A)R function, which may play a role in pathogenesis (Buzzi et al 2006; Chan et al 2006; Gibson et al 2005; Wu et al 2004a, 2006).

Acute administration of GABA yields an increase in the phosphorylation level of the cAMP-responsive element-binding protein in murine hippocampus, but this phenomenon is abolished after repetitive GABA exposure, suggesting desensitization of the signalling pathways and GABA-induced neuroadaptive processes (Ren and Mody 2006). GABA likely plays a key role in synaptic plasticity during development, and GABA in this period may be excitatory (Gibson et al 2006, Owens et al 1996, 1999). GHB may metabolize to GABA and *trans*-4-hydroxycrotonic acid (T-HCA), which is also pharmacologically active at the GABA(B)R and GHBR, respectively (Quang et al 2002). In terms of intracellular signalling, GHB inhibits mitogen-activated protein (MAP) kinase activation via a GABA(B)R-mediated mechanism (Fig. 1). Since MAP kinases mediate numerous physiological changes (e.g. regulation of cell division and differentiation), downregulation of this pathway might occur during GHB intoxication. Additionally, MAP kinases function in long-term neuroadaptive changes following repeated exposure to GHB (Ren and Mody 2003) and perhaps also in myelin expression (Donarum et al 2006).

Glutamine metabolism may also play a role in the pathophysiology of SSADH deficiency (Gibson et al 2003). The major ionotropic Glu receptors are the *N*-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptor. High levels of GHB depress both NMDA and AMPA/kainate receptor-mediated function and may accordingly alter glutamatergic excitatory synaptic transmission (Berton et al 1999). The NMDA receptor antagonist dizocilpine enhances GHB-induced catalepsy in diabetic rats treated with GHB

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