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mTOR Inhibitors Rescue Premature Lethality and Attenuate Dysregulation of GABAergic/Glutamatergic Transcription in Murine Succinate Semialdehyde Dehydrogenase Deficiency (SSADHD), a Disorder of GABA Metabolism

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Abstract

Recent studies have identified a role for supraphysiological GABA in the regulation of mTOR (mechanistic target of rapamycin), a protein kinase with pleiotropic roles in cellular development and homeostasis, including integration of growth factors and nutrient sensing, and synaptic input in neurons (Lakhani et al 2014; Vogel et al 2015). Aldehyde dehydrogenase 5a1-deficient (*aldh5a1*^{-/-}) mice, the murine orthologue of human succinic semialdehyde dehydrogenase deficiency (SSADHD), manifest increased GABA that disrupts mitophagy and increases mitochondria number with enhanced oxidant stress. Treatment with the mTOR inhibitor, rapamycin, significantly attenuates these GABA-related anomalies. We extend those studies through characterization of additional rapalog agents including temsirolimus, dual mTOR inhibitors (torin 1 and 2 (Tor1/ Tor 2), Ku-0063794, and XL-765), as well as mTOR-independent autophagy inducers (trehalose, tat-Beclin 1, FK-506, and NF-449) in *aldh5a1*^{-/-} mice. Rapamycin, Tor1, and Tor2 rescued these mice from premature lethality associated with status epilepticus. XL-765 extended lifespan significantly and induced weight gain in *aldh5a1*^{-/-} mice; untreated *aldh5a1*^{-/-} mice fail to increase body mass. Expression profiling of animals rescued with Tor1/ Tor2 and XL-765 revealed multiple instances of pharmacological compensation and/or correction of GABAergic and glutamatergic receptors, GABA/glutamate transporters, and GABA/glutamate-associated proteins, with Tor2 and XL-765 showing optimal outcomes. Our studies lay the

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Conflict of interest:

Kara R. Vogel declares that she has no conflict of interest.
Garrett R. Ainslie declares that he has no conflict of interest
K. Michael Gibson declares that he has no conflict of interest.

Compliance with Ethics Guidelines

Animal Rights:

All institutional and national guidelines of the care and use of laboratory animals were followed.

Author contributions

Contributed to experimental design and execution: Kara R. Vogel, Garrett R. Ainslie

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groundwork for further evaluation of mTOR inhibitors in *aldh5a1*^{-/-} mice, with therapeutic ramifications for heritable disorders of GABA and glutamate neurotransmission.

Introduction

The inhibitory neurotransmitter GABA derives from excitatory glutamate via the catalytic action of glutamate decarboxylase (*GAD*). Subsequently, GABA converts to succinic acid via the sequential actions of aminobutyrate aminotransferase (*ABAT*) and succinic semialdehyde dehydrogenase (*ALDH5A1*; aldehyde dehydrogenase 5a1=succinic semialdehyde dehydrogenase (*SSADH*)). Mendelian disorders of each catabolic enzyme exist (Pearl et al. 2015; Lapalme-Remis et al. 2015; Rodan et al. 2015). *SSADH* presents with non-specific mild to moderate developmental delay, severe expressive language impairment, variable epilepsy (Parviz et al. 2014; Pearl et al. 2014), and neuropsychiatric problems (i.e., ADHD, OCD, aggression). Metabolically, there is accumulation of glutamate, GABA and the GABA-derivative γ -hydroxybutyrate (GHB; Snead and Gibson 2005), the latter an intermediate with a varied and complex pharmacological history (Maitre et al. 2016) (Fig. 1).

Although historically considered a CNS inhibitory neurotransmitter, a growing literature underscores broader implications for GABA in peripheral roles, as well as in mTOR signaling. Mechanistic target of rapamycin (mTOR) regulates cellular development and homeostasis including integration of growth factors and nutrient sensing, and synaptic input in neurons (Lafourcade et al. 2013; Santinon et al. 2015; Han et al. 2016). For example, mTOR mediates synaptic regulation by modulation of synapse number and miniature inhibitory postsynaptic currents (Weston et al. 2012a). Hyperactive mTOR increases evoked synaptic responses in both glutamatergic and GABAergic neurons, and the glutamatergic component is corrected by the mTOR inhibitor rapamycin. Workman and colleagues demonstrated that GABA_B receptors can activate mTOR via calcium signaling, and further demonstrated that signaling of the GABA_B receptor was necessary for mTOR-dependent protein synthesis (Workman et al. 2013). These few publications highlight the complex synergy that appears to exist between GABA and mTOR.

Lakhani and coworkers recently identified a novel relationship between GABA and autophagy in yeast in which elevated GABA impaired both mitophagy and pexophagy (Lakhani et al. 2014). These findings were extended to the *aldh5a1*^{-/-} mouse, a model for which intervention with rapamycin resulted in a significant mitigation of hepatic elevations of pS6 (a kinase linked to mTOR function), superoxide dismutase (SOD), and mitochondrial number. Comparable impairments of autophagy have recently been documented for vigabatrin, an antiepileptic agent that irreversibly inactivates *ABAT* and elevates GABA (Vogel et al. 2015). Accordingly, the mTOR pathway appears to be a viable therapeutic target for disorders featuring dysregulated GABA homeostasis. To further explore this hypothesis, we have examined the effect of various modulators of mTOR and autophagy in *aldh5a1*^{-/-} mice to further interrogate the preclinical efficacy of this approach. Here, we summarize the outcomes of our studies.

Methods

Reagents and drugs

Rapamycin, Torin 1, Torin 2, Temsirolimus, XL765, Ku-0063794, FK-506, and NF-449 were purchased from Cayman Chemical (Ann Arbor MI) and prepared in DMSO at 25 mg/mL. Trehalose was obtained from Sigma Aldrich (St. Louis MO), and prepared in DMSO at 250 mg/mL. Tat-Bec1 (tat-Bec1) human recombinant peptide was purchased from EMD Millipore (Billerica MA) and prepared at concentrations of 25 and 125 mg/mL. Cell culture grade dimethyl sulfoxide (DMSO) was obtained from Thermo Fisher (Waltham MA).

Animal studies

All animal procedures were approved by the Washington State University IACUC (protocol 4232 and 4276). Tail biopsy of *aldh5a1* mice was performed at DOL 10–12, followed by DNA extraction and genotyping by 3 primer 2 reaction PCR (Hogema et al. 2001). This procedure was repeated at the conclusion of survival studies in order to confirm genotype. For drug treatment, stock solutions were diluted in PBS based on body weight to a total injection volume of 50 μ L. Intraperitoneal injections were given daily between 0700–1000 hours. Rapamycin, Tor1, Tor2, temsirolimus, XL765, Ku-0063794, and FK506 were administered at 5 mg/kg/day; Tor 2 was also further characterized at 10 mg/kg/day. Tat-Bec1 was evaluated at 5 and 25 mg/kg/day, and trehalose at 100 mg/kg/day. Large litters of mice were culled to less than six pups after completion of the first round of genotyping. At weaning (21 days of age), mice were housed with 1–2 identical gender litter mates (not singly). Kaplan-Meier plots of survival data were generated with GraphPad Prism 6, which computed survival proportions and log-rank (Mantel-Cox) test p values, with 0.05 set as the threshold for significance.

Expression Studies

RNA was prepared by pooling liver or brain tissues with n=4 for wild-type (Wt) and mutant (Mt) mice, day of life (DOL) 21, n=2 survivors for Tor1 (DOL 50); n=1 survivor each for 5mg/kg Tor 2, tat-Bec1 and XL-765 (DOL 50), and homogenized with TRIzol® (Invitrogen). The *aldh5a1*^{-/-} model demonstrates uniform lethality by DOL 24 from status epilepticus (Hogema et al. 2001; Gupta et al. 2002), such that survival to DOL 50 is highly significant. Chloroform was added to pooled tissues, and samples were separated by centrifugation to isolate the RNA-enriched aqueous supernatant. RNA was subsequently precipitated and washed with isopropanol and ethanol followed by brief air drying and reconstitution in water. RNA was quantified via absorbance (260 nm) and purity assessed using 260 nm/280 nm absorbance ratio (> 1.8), with storage at -80°C. iScript mastermix (Biorad, Hercules CA) was employed to generate cDNA in an Eppendorf mastercycler per manual instructions. Sybr green (Biorad) reactions (10 μ L) were loaded with 50 ng of cDNA/well into prearrayed and validated, per MIQE guidelines (Bustin et al. 2009), GABA/ Glutamate analysis plates (M394 PrimePCR™ SABioscience target list). A BioRad CFX384 real-time PCR operated with BioRad CFX manager v3.0 software was employed for data acquisition, normalization, expression and statistical analysis with significance set at 0.05 based upon two replicates per group.

Results

Rescue of *aldh5a1*^{-/-} mice from premature lethality – mTOR and dual Inhibitors of autophagy

Ald5ha1^{-/-} mice (mixed gender) provided baseline data, with survival to a maximum of 25 days (n=12, 50% at 17 days). Rapamycin, the classic mTOR complex 1 (mTORC1) inhibitor, extended lifespan of 3 *aldh5a1*^{-/-} mice to 42, 45 and 47 days (n=6, 50% survival to 23 days, 5 mg/kg body weight, p=0.0327*). Temsirolimus, a next generation mTORC1 inhibitor with improved bioavailability, extended lifespan of one mouse to 50 days (n=8, 50% survival at 22–23 days, 5 mg/kg, p=ns) (Fig. 2). Tor1, Tor2 and Ku-0063794 inhibit both complexes of mTOR, mTORC1 and mTORC2. Tor1 extended lifespan for two *aldh5a1*^{-/-} mice to 50 days of life (n=4, 50% survival to 26 days, 5 mg/kg dose, p=0.0116*). Adverse effects observed in *aldh5a1*^{-/-} mice with Tor1 included development of fibrotic tissue at the injection site, ataxia and distended limbs. Tor2 manifested none of these side effects. Tor2 extended lifespan of *aldh5a1*^{-/-} mice, including one mouse at the 5 mg/kg dose (n=8, 50% survival to 19–20 days, p=ns) and two mice at 10 mg/kg (n=5, 50% survival to 25 days, p=0.0074**). Ku-0063794 had no effect on longevity of *aldh5a1*^{-/-} mice (n=8, 50% survival to 22 days, 5 mg/kg, p=ns). *Aldh5a1*^{-/-} mice are essentially static for, or lose, body weight from two weeks of age until expiration (Gupta et al. 2002; Nylen et al. 2008a). XL-765 is a potent dual inhibitor of PI3K and mTOR known to permeate the blood-brain barrier, and its use resulted in extended survival and modest improvement to body weight of *aldh5a1*^{-/-} mice out to 50 days (n=4, 40% survival to 37 days, 5 mg/kg, p=0.0194*).

Rescue of *aldh5a1*^{-/-} mice from premature lethality – mTOR-independent inducers of autophagy

FK-506 (tacrolimus), an inhibitor of calcineurin and an mTOR-independent inducer of autophagy, extended lifespan of *aldh5a1*^{-/-} mice up to 23 days (n=4, 75% survival to 20 days, 5 mg/kg, p=ns). NF-449, a suramin analog and P2X1 inhibitor, induces autophagy through impaired binding of the G protein G_{sa-s}. NF-449 did not extend lifespan (n=5, 60% survival to 22 days, 5 mg/kg, p=ns). Trehalose, a disaccharide which induces autophagy independent of mTOR, extended lifespan of *aldh5a1*^{-/-} mice up to 23 days of life (n=4, 50% survival to 21–22 days, 100 mg/kg, p=ns). Tat-Bec1, an autophagy inducing peptide potentially acting through GAPR-1 (Shoji-Kawata et al. 2013), extended the lifespan of one *aldh5a1*^{-/-} mouse to 50 days (n=5, 50% survival to 17–20 days, 5 mg/kg, p=ns) and one *aldh5a1*^{-/-} mouse to 47 days of life (n=4, 50% survival to 20 days, 25 mg/kg, p=ns) (Fig. 2).

Improved body weight in *aldh5a1*^{-/-} mice via mTOR

In addition to early lethality, *aldh5a1*^{-/-} mice are runted and essentially show little capacity for weight gain from birth until death. The only effective mechanism thus far for induction of weight gain in this animal has been the ketogenic diet (Nylen et al. 2008b). In our study, we monitored weight gain as a function of drug intake (Fig. 3). Fig. 3 presents body mass for the longest lived individual mouse as a function of drug intervention. In our hands, weight gain (best to worst) revealed the following trend: XL-765 > Tor1~ Tor 2~tat-Bec1 > rapamycin.

Gene expression profiling

We assessed the pharmacologic effects of Tor1/ Tor 2, XL-765 and tat-Bec1 on GABA and glutamate receptor subunits and transporters, as well as GABA and glutamate related enzymes and proteins, in the brain. The pooled tissues represented n=4 each untreated *aldh5a1^{+/+}* mice and untreated *aldh5a1^{-/-}* animals (DOL 21), n=2 Tor1 treated *aldh5a1^{-/-}* mice, and one each Tor2, XL-765 and tat-Bec1 treated *aldh5a1^{-/-}* animal. Statistical analysis for the arrays employed a one-way ANOVA with post-hoc Bonferroni *t*-test correction. *Aldh5a1^{-/-}* mice (treated or untreated) were compared to the expression level detected for untreated, control *aldh5a1^{+/+}* mice (expression set to 1.0).

We initially focused attention on GABA receptor subunits and transporters (both vesicular and non-vesicular; Fig. 4). As predicted, for all subunits and transporters, the direction of expression was down-regulation in untreated *aldh5a1^{-/-}* mice, expected with use-dependent effects of elevated GABA. Intervention with only Tor1 led to correction of *Gabrb1* and *Gabrg* expression level. Tor 2, XL-765 and tat-Bec1 were effective in leading to correction of subunit expression, normalizing the levels of *Gabrb3*, *Gabre*, *Slc32a1* and *Slc6a11*. Both Tor1 and Tor 2 resulted in the correction of *Gabra1*, *Gabra2* and *Slc6a12* expression. Tor1, XL-765 and tat-Bec1 normalized *Gabbr1*, and XL-765 and tat-Bec1 also normalized *Gabbr2*. It was of interest that Tor2 did not correct GABA_B receptor (metabotropic) anomalies, while numerous corrections of GABA_A receptors (ionotropic) were observed.

We next turned our attention to glutamate receptors and transporters. The former included kainate, NMDA, AMPA and metabotropic glutamate receptors, while the latter included both vesicular glutamate as well as excitatory amino acid transporters (Fig. 5). As was the case for GABA receptor subunits and transporters, the direction for expression in untreated *aldh5a1^{-/-}* mice was down-regulation, as expected since glutamate is also elevated in this model (Gupta et al. 2004). The sole exception was the up-regulation of the transporter *Slc7a11* (Fig. 5). Intervention with Tor1 corrected the expression of *Gria3*, *Grik5*, *Grin2b*, and *Slc1a1*. Intervention with Tor 2 resulted in the corrected expression of *Gria1*, *Grm7*, *Slc17a6*, *Slc17a8*, and *Slc38a1*. Both interventions lead to the correction of *Gria2*, *Gria4*, *Grik2*, *Grin2b*, *Grin2c*, *Grm1*, *Grm2*, *Grm4*, *Grm5*, *Grm8*, and *Slc1a2*. XL-765 corrected *Grin2b*, *Grm1*, *Grm2*, *Grm5*, *Slc17a6*, *Slc17a8*, *Slc1a1*, *Slc1a2*, and *Slc38a1*. Tat-Bec1 corrected *Grik5*, *Grin2b*, *Grm1*, *Grm6*, *Slc17a6*, *Slc17a8* and *Slc1a2*.

Finally, we extended the preceding studies to include GABA- and glutamate-related enzymes and proteins (Fig. 6). As for the GABA and glutamate receptors and transporters, the direction of expression in untreated *aldh5a1^{-/-}* mice was down-regulation, with only a single instance of upregulation (B2M). Tor2 corrected the aberrant expression of *App*, *Cln3*, *Gls*, *Glul*, *Nsf* and *Snca* (see Fig. 5 for abbreviations). Tor1 corrected the aberrant expression of *B2M*. All the treatments (Tor1, Tor2, XL-765, and tat-Bec1) corrected *Abat*, *Cln3*, *Gad1*, *Gls*, *Glul*, *Gphn*, *Hsp90ab1*, *Nsf*, *Pla2g6* and *Srr*. In some instances, the relationship of these genes to GABA/glutamate metabolism is readily apparent (e.g., *Gls*, glutamate synthetase; *Glul*, glutaminase; *Abat*, aminobutyrate aminotransferase (GABA-transaminase); *Gad1*, glutamic acid decarboxylase), but the relationship of other genes is not immediately obvious. Fig. 7 provides a schematic overview of the interactions for these various proteins and

enzymes between glial cells and the inhibitory GABAergic and excitatory glutamatergic synapses.

Discussion

The current report expands the link between supraphysiological GABA and mTOR signaling described in earlier studies (Lakhani et al. 2014; Vogel et al. 2015). Until now, drugs that have extended the lifespan of *aldh5a1*^{-/-} mice have included taurine, vigabatrin (an irreversible inhibitor of GABA-transaminase), CGP-35348 (a GABA_B receptor antagonist), NCS-382 (a GHB receptor antagonist), and the ketogenic diet (Hogema et al. 2001; Gupta et al. 2002; Nylen et al. 2008b). With the exception of taurine and the ketogenic diet, all target either GABA metabolism, or GABAergic/GHBergic neurotransmission. Taurine was evaluated because of its high content in dam's milk associated with premature lethality of *ald5ha1*^{-/-} mice in the weaning period. Taurine also possesses diffuse GABA_A and GABA_B receptor modulatory effects (Gupta et al. 2002). An open-label trial of taurine in SSADHD patients was, however, without clinical benefit (Pearl et al. 2014). Here, we present new evidence of attenuation of premature lethality in our murine model using mTOR inhibitors, supported by expression array data on GABAergic/glutamatergic systems revealing pharmacological correction with mTOR inhibitors (Donarum et al. 2006).

We found Tor 2, XL-765 and tat-Bec1 to be optimal therapeutic agents for *aldh5a1*^{-/-} mice and that dual inhibition of mTORC1/2 was more effective than mTORC1 inhibition alone. Similar outcomes for survival occurred for tat-Bec1, emphasizing the importance of autophagy activation in the *aldh5a1*^{-/-} mice. Tat-Bec1, not to be confused with exogenous beclin 1 that is an inducer of apoptosis and autophagy (Kang et al. 2011), was the only mTOR-independent autophagy inducer tested (including trehalose, tat-Bec1, FK-506, and NF-449) that increased survival. Similarity of the expression profiles between the dual mTORC inhibitors (Tor2, XL-765) and tat-Bec1 may be related to the feedback mechanism between autophagy and mTOR and inhibitory effects on mTOR during autophagy initiation by the tat-Bec 1 peptide. The adverse effect profile for Tor1 was substantial, even with multiple gene expression corrections (Figs. 4–6). In our hands, these three treatments (Tor 2, XL-765, tat-Bec 1) had no visible effects on the *aldh5a1*^{-/-} phenotype, other than the expected neurological morbidity normally encountered (mild ataxia). Substantial metabolic and neurophysiological data, from our laboratory and others, has previously documented disruptions of the glutamate/glutamine/GABA axis in *aldh5a1*^{-/-} mice, in addition to abnormalities of GABA_A and GABA_B receptors. Early metabolic studies verified elevations of GHB, GABA, and glutamate (Gupta et al. 2002; Gupta et al. 2004) that correlated with alterations of glutamate/glutamine cycling in the CNS (Chowdhury et al. 2007). Neurophysiological and neurochemical characterization of *aldh5a1*^{-/-} mice verified central, as well as localized (hippocampal, cortical) disruption of GABAergic neurotransmission that manifested as down-regulated GABA_B and GABA_A receptors (Cortez et al. 2004; Wu et al. 2006; Buzzi et al. 2006; Drasbek et al. 2008; Dósa et al. 2010; Vardya et al. 2010; Errington et al. 2011). The data in mice have been confirmed in SSADHD patients using transcranial magnetic stimulation for GABA_B receptors (Reis et al. 2012) and ¹¹C-flumazenil binding for GABA_A receptors (Pearl et al. 2009), as well as magnetic resonance spectroscopy (Ethofer et al. 2004; Wang et al. 2015) with spectral editing for GABA and Glx

(Glx=glutamine and glutamate combined). The current results, indicating significant down-regulation of receptor subunits and transporters, is consistent with the mouse and human neurophysiological/neurochemical data, but the pharmacological compensation with mTOR inhibitors, is quite novel and clinically relevant (Figs. 4–6).

An exciting finding in our study was the effect of Tor1 and Tor 2 on glutamatergic systems (Fig. 5), which was unexpected despite our previous demonstration of glutamate increase in the CNS of *aldh5a1*^{-/-} mice (Gupta et al. 2004). A link between glutamatergic signaling and mTOR activation is beginning to emerge (Weston et al. 2012b; Theilhaber et al. 2013; Chen et al. 2015; Rosenberg et al. 2015). These observations are relevant to *aldh5a1*^{-/-} embryonic mice, in which neurotransmitter imbalances are evident as early as E13, a developmental period in which GABAergic neurotransmission is still excitatory with the potential for development of a heightened excitatory state that may predispose neural networks to epilepsy in the embryonic period (Jansen et al. 2008). Our results for pharmacological compensation of glutamatergic systems with Tor1 and Tor 2 suggest it will be worthwhile to investigate glutamatergic neurophysiology in *aldh5a1*^{-/-} mice as a readout of treatment efficacy, an area which has been underexplored in this animal model.

We found that mTOR-independent activators of autophagy were far less effective in rescue of *aldh5a1*^{-/-} mice from premature lethality (Fig. 2), with the exception of tat-Bec1. Conversely, dual mTOR pathway inhibitors appeared to be superior to mTOR complex 1 (mTORC1) inhibitors in facilitating weight gain and longevity (Fig. 3). Moreover, the data of Fig. 6 were of interest with respect to broader pathway compensation for *aldh5a1*^{-/-} mice treated with Tor1 and 2, revealing robust correction of the down-regulated expression of *Abat*, *Gad1*, *Gls* and *Glul* (Fig. 4). The relationship(s) of many of the GABA- and glutamate-associated proteins and genes to GABAergic/glutamatergic signaling is schematically diagrammed in Fig. 7. Two genes quantified in the array data of Fig. 5 were of additional interest. The down-regulation of both *Adora2a* and *Avp* were significantly exacerbated in Tor1 animals by ~ 50- to 60-fold. The adenosine A2A receptor (*Adora2a*) broadly functions in GABA and glutamate release (heteroreceptors), while arginine vasopressin (*Avp*) is the precursor of antidiuretic hormone (Vaz et al. 2015). This observation in the Tor1 group on *Avp* may have underpinned some of the adverse effects we observed with this mTORC1/2 inhibitor. Nevertheless, these two results would strengthen the argument for not using Tor1 intervention in *aldh5a1*^{-/-} mice. It is of interest that the accumulation of GABA in both *aldh5a1*^{-/-} mice alters arginine metabolism and induces accumulation of guanidinobutyrate and guanidinoacetate (Jansen et al. 2006), which may provide some insight into alterations of arginine vasopressin (*Avp*).

In summary, the results presented provide evidence for pharmacological compensation of GABAergic/glutamatergic networks in *aldh5a1*^{-/-} mice treated with the mTOR inhibitors Tor1 and Tor 2. The clinical relevance of these findings for treatment of SSADHD are significant, since it is likely that multiple treatment approaches will be needed to gain incremental improvements in the phenotype. Nonetheless, the potential of mTOR inhibitors to compensate transcriptional abnormalities in central GABAergic/glutamatergic pathways is an attractive consideration in the therapeutics of SSADHD. The translational relevance of this finding is high, since mTOR inhibitors (primarily rapamycin and temsirolimus) are

actively under study in clinical trials (www.clinicaltrials.gov). More broadly, our data supports consideration of mTOR inhibitors in other Mendelian disorders, including GABA-transaminase deficiency (Pearl et al. 2015), an expanding disorder of GABA metabolism, as well as disorders of glutamate receptors (Morice et al. 2013; Soto et al. 2014). Future studies in *aldh5a1*^{-/-} mice will further gauge correction of the phenotype of *aldh5a1*^{-/-} mice with mTOR inhibitors using electrocorticographic assessment (Cortez et al. 2004). These efforts will likely include more complete metabolic profiling of key metabolites (e.g., GABA, SSA, succinate, γ -hydroxybutyrate) as well as further evaluation of candidate clinical biomarkers.

Acknowledgments

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Abbreviations

GABA

gamma-aminobutyric acid

Glu

glutamate

Tor1

Torin 1

Tor 2

Torin 2

SSADH

succinic semialdehyde dehydrogenase

SSADHD

succinic semialdehyde dehydrogenase deficiency

aldh5a1 (identical to succinic semialdehyde dehydrogenase)

aldehyde dehydrogenase 5a1

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Synopsis

Dual mTOR complex 1/2 and PI3K inhibitors extend lifespan, improve bodyweight and correct GABA/glutamate receptor expression profiles in SSADH-deficient mice.

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Fig. 1. Interrelationships of GABA metabolism

Arrows (downward/upward) indicate the direction of metabolite disruption in *aldh5a1*^{-/-} mice. Abbreviations: Gln, glutamine; Glu, glutamate; GABA, 4-aminobutyrate; SSA, succinic semialdehyde; GHB, gamma-hydroxybutyrate; mTOR, mechanistic target of rapamycin; GAD, glutamic acid decarboxylase; GABA-T, GABA-transaminase; SSR, succinic semialdehyde reductase; GHBDH, gamma-hydroxybutyrate dehydrogenase; SSADH, succinic semialdehyde dehydrogenase (site of the defect in patients with SSADHD); GLS, glutamate synthetase; GLNASE, glutaminase. Vigabatrin (VGB), an antiepileptic and irreversible inhibitor of GABA-T, is a frequently employed therapeutic agent for SSADHD.

Fig. 2. Modified Kaplan-Meier survival curves for *aldh5a1*^{-/-} mice

Top, mTOR inhibitors; middle, mTOR-independent inhibitors of autophagy; bottom, mixed (dual) mTOR inhibitors. P-values indicated in this figure correspond to the overall data plotted on each pane for each class of inhibitor. For drug specific statistical evaluations, please see text.

Fig. 3. Anthropomorphic characteristics (body weight) of longest surviving animals undergoing therapy

Smoothed trendline characteristics of daily body weight with treatment intervention (RM, rapamycin; Tor1, torin 1; Tor2, torin 2).

Fig. 4. Gene array expression data for GABA receptors and transporters in the brain
 GABA_A and GABA_B receptor subunits and names, as well as GABA transporters, are shown above individual graphs. Expression in wild-type mice is set to 1.0. For methodological and statistical details, see text.

Fig. 5. Gene array expression data for glutamate receptors and transporters in the brain Glutamate receptor (AMPA, kainate, NMDA and metabotropic) subunits and names are shown above individual graphs. Expression in wild-type mice is set to 1.0. For methodological and statistical details, see text.

Fig. 6. Gene array expression data for GABA and glutamate related enzymes and proteins in the brain

Gene names are shown above individual graphs. Expression in wild-type mice is set to 1.0. For methodological and statistical details, see text. Fig. 7 provides a schematic that clarifies the role of these genes/receptors/enzymes (putative and proven) in both glutamatergic and GABAergic signaling.

Fig. 7. Schematic of the interrelationships of glial cell function with that of glutamatergic and GABAergic synapses

The roles of *Aldh5a1* (localized to the mitochondria, not shown), *Abat* (4-Aminobutyrate Aminotransferase), *Gad1* (glutamate decarboxylase 1), *Gls* (glutaminase) and *Glul* (ferredoxin-dependent glutamate synthase 1) are recognizable. Other genes characterized in array expression (Fig. 4) data and whose role(s) are less readily apparent in glial-neuronal signaling include (circled in the figure): *Adora2a* (adenosine A2A receptor), a heteroreceptor modulating glutamate/GABA release; *App* (amyloid beta precursor protein), a soluble oligomeric protein interacting with NMDA receptors; *Avp* (arginine vasopressin), a hormone active in stimulation of regional GABAergic neurons; *Cln3* (ceroid lipofuscinosis 3), a gene shown to perturb glutamine and glutamate levels in cortex and cerebellum; *Gphn* (gephyrin), a scaffold protein stabilizing GABA_A receptors; *Itpr1* (inositol 1,4,5-trisphosphate receptor1), a phospholipase receptor with interactions targeting the metabotropic glutamate receptor (mGluR); *Nsf* (*N*-ethylmaleimide-sensitive factor), a protein interacting with AMPA-type glutamate receptors; *Pla2g6* (phospholipase A2 group VI), one of multiple phospholipases regulating the release of aspartate glutamate, glycine and GABA; *Snca* (synuclein alpha), a secreted protein interacting with ATP-sensitive potassium channels modulating GABA transmission; and *Srr* (serine racemase), an activator of NMDA receptors. Not shown are β -2-microglobulin (*B2M*) and heat shock protein 90kDa alpha class B member 1 (*Hsp90ab1*), both of which may exert chaperone-type roles in scaffolding of oligomeric glutamatergic and GABAergic receptors.