Potential drug targets for SLC6A1

Ana C. Puhl, PhD
Collaborations Pharmaceuticals

We bring novel and repurposed therapies from early research to the clinic with collaborators / partners in a quasi-virtual model

- Founded in 2015. 9 employees
- 6 FDA Orphan Drug Designations
- > $7M of funding to date on 16 NIH, DOD and DTRA-funded projects on developing drugs for neglected and rare diseases as well as software technologies
We focus on finding treatments for rare and neglected diseases

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* **italics** = biologic  
* **optioned Vanderbilt Univ.**
Machine Learning

Data

Screen/Filter

Build machine learning models using Assay Central

Cherry pick compounds Assay Central

Test: using melting temperature

Confirm hits: enzyme activity and binding assays

Test chaperones in patient cell lines

ChEMLB

PubChem

Binding DB

Perform Experiments

Build Models

Curate Data

Generate Predictions

FDA approved drugs and other libraries

Do these molecules cross BBB?

Cytotoxicity?

Collaborators

In house

What do we know about SLC6A1?

**GAT-1 Knockout Mice Exhibit Spontaneous Spike-Wave Discharges (SWDS) and Absence Seizures**


**De Novo Inactivating Variants in SLC6A1 Were Reported in Up to 4% of Patients with Myoclonic Atonic Epilepsy (MAE), Suggesting That Pathogenic SLC6A1 Variants Might Be Specific for MAE**


**MAE Is a Syndrome Characterized by the Presence of Myoclonic-Atonic Seizures, Usually in an Otherwise Normal Child, Which May Typically Develop Cognitive Impairment After Seizure Onset**

GAT-1 dysfunction

- GAT-1 dysfunction is expected to reduce GABA clearance, leading to increased GABA levels, both at the synapse and extrasynaptically.
- Could lead to the overstimulation of extra synaptic GABA$_A$ and GABA$_B$ receptors.
Hypothesis about the disease mechanism

Increased $GABA_A$-mediated tonic inhibition can lead to neuronal hyperpolarization and burst pattern firing in thalamocortical neurons, which can promote the generation of spike-wave discharges.

Similarly, prolonged activation of $GABA_B$ receptors is known to stimulate low voltage-activated (T-type) $Ca^{2+}$ channels, which can cause recurrent excitation within the thalamocortical system through successive $Na^+$ spikes.

$GABA_B$ receptor activation causes absence seizures in mice and rats and that pretreatment with a $GABA_B$ antagonist can decrease the duration of chemically induced absence seizures.

Reduced GAT-1 function could also decrease the amount of intracellular $GABA$ available for release to activate $GABA_A$-mediated synaptic (phasic) signaling. Decreased $GABA_A$-mediated synaptic signaling is already associated with variants in several $GABA_A$ receptor subunits in genetic epilepsies.

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Together, these results suggest that reduced GAT-1 function might lead to epilepsy through overactivation of extra synaptic GABA_A and GABA_B receptors, and reduction in GABA_A synaptic signaling.
Potential drug targets for SLC6A1
Antagonists and GABA$_B$ receptors

- GABAB receptors can produce a late hyperpolarization in response to synaptically released GABA by enhancing K+ permeability through G-protein-coupled inwardly rectifying potassium channels (GIRK/Kir3.x)

- They can activate low voltage-activated (T-type) Ca$^{2+}$ channels, which can cause recurrent excitation within the thalamocortical system
A Role for Diminished GABA Transporter Activity in the Cortical Discharge Phenotype of MeCP2-Deficient Mice

Liang Zhang¹,²,³, Robert G Wither⁴,⁵, Min Lang²,⁴,⁵, Chiping Wu¹,⁴, Elena Sidorova-Darmos⁴,⁵, Hristo Netchev⁴, Catherine B Matolcsy⁴, Orlando Carter Sneed²,³ and James H Eubanks⁶,²,⁴,⁵,⁶

¹Division of Fundamental Neurobiology, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; ²University of Toronto Epilepsy Research Program, University of Toronto, Toronto, ON, Canada; ³Department of Medicine (Neurology), University of Toronto, Toronto, ON, Canada; ⁴Division of Genetics and Development, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; ⁵Department of Physiology, University of Toronto, Toronto, ON, Canada; ⁶Department of Surgery (Neurosurgery), University of Toronto, Toronto, ON, Canada

• Loss-of-function mutations of methyl-CPG-binding protein 2 (MECP2) can cause Rett syndrome
• GAT-1 knockout mice and MeCP2- deficient mice display overlapping phenotype
• GAT-1 Protein Levels, but not GABAB or Extra-Synaptic GABAA Receptor Subunits, are Diminished in the MeCP2-Null Cortex
• Enhancing GABAB Receptor Activity Increases Cortical Epileptiform Discharges in Mecp2+/− Mice
• Attenuating GABAB Receptor Activity Decreases Cortical Epileptiform Discharges in Mecp2+/− Mice
GABA_B receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures

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Abstract

Chronic atypical absence seizures are a component of the Lennox–Gastaut syndrome, a disorder invariably associated with severe cognitive impairment in children. However, the cause of this intellectual delay remains unclear. The AY9944 model of chronic atypical absence seizures in rats reliably reproduces the electrophoretic, behavioral, pharmacological and cognitive features of clinical atypical absence. Using this model, we tested the hypothesis that the cognitive impairment associated with this disorder involves a γ-aminobutyric acid B (GABA_B) receptor-mediated mechanism. Therefore, we examined the effect of a specific, high affinity GABA_B receptor antagonist, CGP35348, on the atypical absence seizures, the working memory deficits, and the altered long-term potentiation that we have observed in the AY9944 model. CGP35348 blocked atypical absence seizures, restored long-term potentiation to normal level, and reversed the cognitive deficit in the AY9944-treated animals. However, dose–response studies showed that lower doses of CGP35348 that failed to influence atypical absence seizure activity, completely reversed the spatial working memory deficit. These data suggest that GABA_B receptor-mediated mechanisms are responsible for the cognitive dysfunction in the AY9944 model of chronic atypical absence seizures and further, that their cognitive impairment is independent of the seizure activity. The data raise the possibility that GABA_B receptor antagonists may have therapeutic potential for the treatment of cognitive impairment in epilepsy syndromes where atypical absence seizures are a component.

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Keywords: GABA_B receptor; CGP35348; Atypical absence seizure; Cognitive deficit; Radial arm maze
Modulators of GABA A receptor

Tonic GABA_A Receptor-Mediated Signaling in Epilepsy
Matthew C Walker and Dimitri M Kullmann* 1

• GABAA receptors can mediate a “tonic” form of signaling that is not time-locked to presynaptic action potentials, and which depends upon detection of ambient GABA by extrasynaptic receptors.
• Tonic currents can have a paradoxical excitatory role
• Tonic currents hyperpolarize thalamocortical neurons and so modulate their firing pattern from regular to burst firing.
• Tonic currents are increased in animal models of absence epilepsy, and promote the generation of spike-wave discharges
Cortical astrocytes express GAT-1 and GAT-3 subtypes, and it has been estimated that ~20% of extracellular GABA may be taken up into astrocytes. We found $A_1R-A_2AR$ receptor heteromers in astrocytes. $A_2AR$ protomer mediating facilitation of GABA transport into astrocytes.
Brain-derived Neurotrophic Factor (BDNF) Enhances GABA Transport by Modulating the Trafficking of GABA Transporter-1 (GAT-1) from the Plasma Membrane of Rat Cortical Astrocytes

Sandra H. Vaz1,2, Trine N. Jørgensen3, Sofia Cristóvão-Ferreira1,2, Sylvie Duflot1,5, Joaquim A. Ribeiro1, Ulrik Gether4 and Ana M. Sebastião1,5,1

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Capsule

Background: Transport of GABA into astrocytes is crucial for excitability control.

Results: The neurotrophin BDNF, through TrkB receptor activation, enhances GABA transport into astrocytes, which requires adenosine A2A receptor signaling.

Conclusion: BDNF plays an active role in the synaptic clearance of GABA.

Significance: This new regulatory role for TrkB receptors discloses their relevance for excitability control at the tripartite synapse.

Enhance GAT1 GABA uptake

BDNF enhances GAT-1 GABA transport in astrocyte primary cultures.

BDNF (10 ng/ml) | - | + | - | + | - | - | + | - 

BDNF (30 ng/ml) | - | - | + | - | - | - | - | -
Inhibitors of Vesicular GABA transporter (VGAT)

• VGAT fuse with the presynaptic membrane of the cell to release GABA from the presynaptic cell to the synaptic cleft
Questions to address

• Amount of GABA in synaptic cleft compared to WT

• Which receptors are upregulated in this disease?
  • Evaluate which GABA targets are upregulated or downregulated by the excess of GABA in the synaptic cleft.
  • Which receptor has been upregulated: $GABA_A$, $GABA_B$?
    This information might be useful to define better targets.

• Study the pharmacological mechanism of seizures
  • Not enough inhibitory signal, since there is less GABA phasic release?
  • Low voltage-activated (T-type) $Ca^{2+}$ channel, activated by GABA B receptor?
  • Activation of other receptors?
Ongoing research

• 2 compounds:
  • CPI1204
  • CPI1205

• Therapeutic effect in cultured neurons, astrocytes
• GABA levels in lysates
• GABA receptors expression
• Mice Model: SLC6a1\textsuperscript{+/A288V}
• Thermal induction - EEG recording
Next steps

- Test in mouse model SLC6A1+/A288V

- Use machine learning to build models

- Select compounds - prioritize FDA approved drugs

- Test if compounds can prevent seizures in Zebrafish model of the disease
Thank you!

Collaborations Pharmaceuticals, Inc.

No disease is too small

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