Shah *et al.*: *Intrinsic Activity*, 2023; **11**(Suppl.1):A2.27
doi:10.25006/IA.11.S1-A2.27
published online: 22 September 2023

INTRINSIC ACTIVITY

# 27th Scientific Symposium of the Austrian Pharmacological Society

Vienna, 29–30 September 2023

*MEETING ABSTRACT*

## A2.27

## How do missense mutations of a highly conserved glycine residue in the human GABA transporter 1 trigger epilepsy?

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**Background:** The human γ-aminobutyric acid (GABA) transporter 1 (hGAT-1) belongs to the solute carrier 6 (SLC6) gene family. It mediates neurotransmission by rapidly clearing GABA from the synapse into neurons and astrocytes. Copious point mutations in hGAT-1 have been associated with developmental delay, myoclonic-atonic and generalized epilepsies, autism and intellectual disability. Many of these mutants are known to impair protein folding, causing their retention in the endoplasmic reticulum (ER) and precluding their proper delivery to the cell surface. Folding defects can be corrected by treatment with chemical and pharmacological chaperones. Our aim is to probe the molecular features of two novel hGAT-1 disease variants in HEK 293 cells (*in vitro*), as well as in primary neuronal cultures and *Drosophila melanogaster* (*in vivo*).

**Methods:** The cellular localization and expression distribution of the wild-type hGAT-1 and two epilepsy variants thereof, G443D and G443V (created by site-directed mutagenesis), were investigated in transiently transfected HEK 293 cells. Their deglycosylation profiles were studied by immunoblotting using endoglycosidase H (Endo H). Confocal microscopy was utilized to examine the subcellular localization of the individual GATs, tagged with yellow fluorescent proteins (YFP) at their amino-terminal domains. Cyan fluorescent protein (CFP)-tagged calnexin was employed as an ER marker, while trypan blue delineated plasma membrane compartments. Functional consequences of the above mutations were examined by radiotracer GABA uptake assays, to determine the Michaelis–Menten kinetic parameters ($K_m$ and $V_{max}$), and ultimately the potential of various small molecules to rescue the variants by pharmacochaperoning.

**Results:** Deglycosylation experiments revealed distinct expression patterns for the wild-type hGAT-1 and the two G443 variants. In short, ER-resident proteins are core-glycosylated (and sensitive to Endo H) whereas proteins located at the plasma membrane are mature-glycosylated (and hence resistant to Endo H). Accordingly, two protein bands were observed for the wild-type hGAT-1. The G443V variant presented only core-glycosylated species, contrasting to G443D, which also exhibited some mature-glycosylated bands. Confocal microscopy substantiated these findings: the mutants accumulated in the cell interior, co-localized with the ER-resident chaperone calnexin. Wild-type hGAT-1, on the other hand, was correctly targeted to the plasma membrane, overlapping with the trypan blue staining. We thus infer that both variants happen to be partially-to-fully misfolded and trapped in the ER compartment. Additional studies are underway to elucidate the functional consequences of both G443V and G443D mutations on GABA uptake.

**Discussion:** Upon dysfunction of plasmalemmal GATs, presynaptic GABA pools decline, thus perturbing the ensuing phasic neurotransmission. Concomitantly, there is a rise in extrasynaptic GABA levels, which in turn impact extrasynaptic $GABA_A$ and $GABA_B$ receptors to induce tonic inhibition. Since most of the reported pathogenic hGAT-1 variants are loss-of-function/misfolded transporters, their clinical manifestations are severe. Small molecules, such as the chemical chaperone 4-phenylbutyrate (4-PBA), liothyronine and the specific hGAT-1 blocker tiagabine, can be beneficial in mending the folding and activity of such variants. We plan to extrapolate our ongoing experiments to hippocampal neurons, and further translate them to *in vivo* studies in *Drosophila melanogaster*. Collectively, these data ought to impart mechanistic acumens crucial for developing effective therapeutic options for hGAT-1-linked syndromes.

**Acknowledgements:** This work was supported by the Austrian Science Fund FWF (project no. P36574-B27) to S.S.

**Keywords:** human GABA transporter 1 (hGAT-1) – γ-aminobutyric acid (GABA) – epilepsy – protein folding – pharmacochaperoning

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