

24<sup>th</sup> Scientific Symposium of the Austrian Pharmacological Society  
Graz, 27–28 September 2018

MEETING ABSTRACT

A3.7

**4-Phenylbutyrate corrects folding-deficient creatine transporter-1 variants associated with the creatine deficiency syndrome**

Sonja SUCIC\*, Ali EL-KASABY and Michael FREISSMUTH

*Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University of Vienna, Austria*

**Background:** The human creatine transporter 1 (hCRT-1, SLC6A8) is a member of the sodium-dependent neurotransmitter transporter family. Creatine transporter deficiency (CTD) has been associated with a number of disorders, ranging from epilepsy to mental retardation, autism, development delay, behavior problems, motor dysfunction to gastrointestinal symptoms. Diseases arising from misfolding of other proteins belonging to the SLC6 protein family have been reported in the literature; e.g. mutations in the dopamine transporter (DAT) cause infantile parkinsonism/dystonia. In CRT-1, sixteen mutations have been linked to the creatine deficiency syndrome in people [1]. One of these variants is a conservative mutation G132V, associated with severe mental retardation in children. Interestingly, the mutation of an equivalent glycine residue in *Drosophila melanogaster* DAT (G108Q-dDAT) leads to a sleepless phenotype in flies [2]. In the present study we examined the molecular basis of CTD-associated mutations and the pharmacological means by which these can be functionally recouped.

**Methods:** We generated 16 CTD-causing mutations in hCRT-1 by site-directed mutagenesis (Quickchange, Stratagene). All mutants were pharmacologically characterised by performing [<sup>3</sup>H]creatine assays, as well by confocal microscopy and biochemical techniques.

**Results:** By creatine transport activity assays, we showed that most CTD mutants have less than 5% of the activity of the wild-type hCRT-1. Confocal microscopy experiments showed that the mutants are retained in the endoplasmic reticulum (ER) compartment, in a complex with an endogenous folding sensor calnexin, whereas the wild-type transporter reaches the cell surface. Using immunoblotting of detergent lysates, the mature fully-glycosylated band was only present in the wild-type hCRT-1 samples. Upon treatment with 4-phenylbutyrate, the surface expression and uptake activity of several mutants was restored.

**Discussion:** Treatment with the chemical chaperone 4-phenylbutyrate ought to be an effective therapy for some CTD patients. This work is clinically relevant and ought to grant promising therapeutic options eagerly awaited for by CTD patients worldwide.

**Acknowledgements:** This work was supported by the Austrian Science Fund FWF (project no. P31255-B27 to S.S.).

**References**

1. Asjad HMM, Kasture A, El-Kasaby A, Sackel M, Hummel T, Freissmuth M, Sucic S: **Pharmacochaperoning in a *Drosophila* model system rescues human dopamine transporter variants associated with infantile/juvenile parkinsonism.** *J Biol Chem*, 2017; 292(47):19250–19265. doi:10.1074/jbc.M117.797092

2. Kasture A, El-Kasaby A, Szöllösi D, Asjad HMM, Grimm A, Stockner T, Hummel T, Freissmuth M, Sucic S: **Functional Rescue of a Misfolded *Drosophila melanogaster* Dopamine Transporter Mutant Associated with a Sleepless Phenotype by Pharmacological Chaperon.** *J Biol Chem*, 2016; 291(40): 20876–20890. doi:10.1074/jbc.M116.737551

\*Corresponding author e-mail: [sonja.sucic@meduniwien.ac.at](mailto:sonja.sucic@meduniwien.ac.at)