El-Kasaby *et al.: Intrinsic Activity*, 2016; **4**(Suppl.3):A4.1
http://www.intrinsicactivity.org/2016/4/S3/A4.1
published online: 5 September 2016

22nd Scientific Symposium of the Austrian Pharmacological Society: Joint Meeting with the Hungarian Society for Experimental and Clinical Pharmacology

Vienna, 8–10 September 2016

MEETING ABSTRACT

A4.1

Functional rescue of misfolded SLC6 transporters

Ali El-KASABY, Ameya KASTURE, H.M. Mazhar ASJAD, Michael FREISSMUTH and Sonja SUCIC*

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

Background: The physiological role of proteins belonging to the solute carrier 6 (SLC6) family is to transport neurotransmitters, amino acids, and osmolytes such as betaine, taurine, and creatine. Their dysfunction has been linked to different neurological and psychiatric disorders. SLC6 transporters are proteins of twelve membrane-spanning helices and cytosolic amino and carboxy termini. Mutations in the coding sequences of SLC6 transporter genes are known to cause protein misfolding. The aim of our study is to characterise and rescue misfolded/dysfunctional SLC6 transporter mutants using pharmacological chaperones.

Methods: Mutations of interest were created by site-directed mutagenesis using the QuikChange™ kit (Stratagene). HEK 293 or Schneider cells were transfected with plasmids encoding the wild-type and mutant transporters using Lipofectamine 2000 (Invitrogen) and Effectene (Qiagen), respectively. Radioligand uptake, confocal laser scanning microscopy and immunoprecipitation experiments were performed to study the mutations on molecular level. dDAT-G108Q flies were treated with pifithrin-μ and noribogaine and total sleep was quantified to assess the effects of pharmacochaperoning.

Results: Mutations in *Drosophila melanogaster* DAT (dDAT) abolish dopamine uptake and lead to a sleepless phenotype in flies. We showed that a single-point mutation in dDAT (dDAT-G108Q) arises due to defective protein folding. The mutant exhibited no specific dopamine uptake in HEK 293 or Schneider cells. Moreover, dDAT-G108Q was retained in the endoplasmic reticulum (ER), which was illustrated both by confocal microscopy and co-localisation with the ER-resident chaperone calnexin. We also found that dDAT-G108Q associates with significantly higher levels of HSP70-1A and calnexin, compared to the wild-type dDAT. This interaction was markedly reduced upon treatment with noribogaine or pifithrin-μ. A combined action of the two compounds resulted in an additive effect, *i.e.* enhanced surface expression. The pharmacochaperoning action of noribogaine can be accounted for by its ability to stabilise the inward-facing conformation of DAT, whereas pifithrin-μ acts by inhibiting HSP70. Noribogaine and pifithrin-μ also restored sleep *in vivo* in dDAT-G108Q-expressing flies. Interestingly, in the human creatine transporter-1 (hCRT-1), a conservative mutation of the equivalent glycine residue (hCRT-1-G132V) is linked to severe mental retardation in children. We found that this mutant has approximately 10% of the activity of the wild-type hCRT-1, and confirmed that the dramatic loss of function is also due to protein misfolding and retention in the ER compartment.

Discussion: Evidently, this particular glycine residue plays a common, crucial role in protein folding in a variety of members of the SLC6 family. In fact, the glycine is absolutely conserved among all SLC6 transporters. It resides in the first intracellular loop, juxta-membrane to the second membrane-spanning helix, a region already known to be involved in the folding of the related serotonin transporter [1].

Acknowledgements: This work was supported by FWF projects SFB 35-10 (to M.F.) and P 27518-B27 (to S.S.).

Reference

1. Koban F, El-Kasaby A, Häusler C, Stockner T, Simbrunner BM, Sitte HH, Freissmuth M, Sucic S: **A salt bridge linking the first intracellular loop with the C terminus facilitates the folding of the serotonin transporter.** *J Biol Chem,* 2015; 290(21):13263–13278. doi:10.1074/jbc.M115.641357

*Corresponding author e-mail: *sonja.sucic@meduniwien.ac.at*