

FUNCTIONAL CHARACTERIZATION OF A NOVEL ARGININE EXCHANGER FROM TRYPANOSOMA CRUZI

Henriques, C¹; Fairman, WA²; Catanho, M¹; Degrave, WM¹ and Amara, SG²

¹Departamento de Bioquímica e Biologia Molecular, IOC- FIOCRUZ, Rio de Janeiro-RJ, Brazil; ²Department of Neurobiology, University of Pittsburgh, Pittsburgh- PA, USA

Trypanosoma cruzi the aetiological agent of Chagas disease undergoes a series of morphological and physiological adaptations to survive within the insect and mammalian hosts. Thus, transporters can act as sensors of nutrient availability, play a role in cellular homeostasis and metabolism. *T.cruzi* can not synthesize arginine, they have to get arginine from the environment. Therefore, our purpose was the functional characterization of a novel arginine transporter, *TcCAT1.1*, in heterologous systems. Interestingly, *TcCAT1.1* displayed lower identity to human arginine transporters (hCAT), 10% identity and 23% similarity at the aminoacid level, when compared to N- system NAT-1. Substrate saturation curves were performed in *S.cerevisiae* and an apparent K_m of $85 \pm 36,37 \mu\text{M}$ was inferred for [³H]-arginine. Competition assays were performed with 100 fold competitor over [³H]-arginine. From the compounds tested, uptake was significantly inhibited by canavanine (70%). Ornithine, showed low affinity for *TcCAT1.1*, 10% inhibition of [³H]-arginine uptake, and K_m of $1,7 \pm 0,24 \text{ mM}$, similar to other cationic aminoacids. Trans-stimulation was observed in *X.laevis* oocytes expressing *TcCAT1.1* pre-loaded with arginine, whose [³H]-arginine uptake increased 7 fold. Oocytes pre-loaded with [³H]- arginine displayed 16 fold higher efflux of [³H]-arginine, than control. *T.cruzi* super-expressing *TcCAT1.1* and *GFP-TcCAT1.1* are being generated to explore the exchanger mechanism and localization into the protozoa.

Supported by CNPq; FAPERJ; University of Pittsburgh

Key words: Arginine, transporters; *T.cruzi*