Marco NIELLO

Thesis Supervisor: Harald SITTE, Medical University of Vienna, Institute of Pharmacology

Co-Supervisor: Margot ERNST, Medical University of Vienna, Department of Molecular Neurosciences

MolTag student, 2nd Funding Period

Defense: December 2019

PhD Thesis title: „Pharmacological characterization and selectivity profile of different synthetic cathinones among monoamine transporters"

ABSTRACT

New psychoactive substances (NPS) are variants of pharmaceuticals or known drugs of abuse developed to retain psychoactive effects and circumvent legal restrictions, resulting in a public health threat. Synthetic cathinones are a common group of NPS. Methcathinone (MCAT) is a NPS and derivative of methamphetamine, which has been a very popular drug of abuse during the ´80. Methcathinone targets the dopamine-, norepinephrine- and serotonin-transporters (DAT, NET and SERT respectively). Under physiological conditions, DAT, NET and SERT re-uptake monoamines after quantal-release, controlling the level of neurotransmitters available for binding the synaptic receptors. Compounds acting at monoamine transporters can be classified in two main groups. Cocaine-like drugs are non-transported inhibitors which bind to the transporters and stabilize the transporter into the outward-open conformation. Amphetamine-like drugs, or releasers, are instead translocated into the cytosol by the transporter. Once in the cytosol, they disrupt the vesicular storage and target several kinases. However, in the recent past, more complex mechanisms of action have been found. These include atypical inhibition, and partial release. Atypical inhibition refers to the stabilization of the transporter in other conformations than the outward-facing conformation. Partial release refers to the ability of a substrate to elicit a blunted efflux when compared to classical releasers such as *para*-chloro-amphetamine (PCA) or D-amphetamine. Despite their mechanism of interaction with

the transporters, so far, compounds preferring DAT over SERT displayed higher abuse risk than those preferring SERT. In fact, SERT-selective drugs have also shown higher therapeutic outcome in comparison to the DAT-selective ones. Thus, understanding how to convert DAT-selective releasers into SERT-selective releasers may provide new classes of molecules with potential interest for treating neuropsychiatric disorders. Recently, *para*-substitution of methcathinone has been shown to largely change the selectivity between DAT and SERT. Compounds carrying a larger *para*-substituent are more SERT-selective than those with a smaller *para*-substituent. We have tested different *para*-substituted MCATs using biochemical, computational and electrophysiological approaches. We have found that other and more complex features of the *para*-substituent than its size underly the conversion of a DAT-selective to a SERT-selective substrate. In addition, our experiments suggested that *para*-Trifluoromethyl-methcathinone (pCF_3-MCAT) can trap a fraction of SERTs in an inactive state by occupying the allosteric site. Because on the remaining SERT-fraction pCF_3-MCAT act as substrate, the overall result is a partial release. These findings indicate the possibility of an alternative, and so far, undescribed mechanism of action for partial releasers which involves the allosteric site of SERT. Our observations emphasize that the substrate permeation pathway of monoamine transporters supports multiple binding modes, which can be exploited for drug design.