

Nanopharmacological force sensing on monoamine transporters

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Monoamine transporters (MATs) consist of serotonin transporter (SERT), dopamine transporter (DAT), and norepinephrine transporter (NET), which are neurotransmitter sodium symporters. MATs reuptake the released monoamines from the synaptic cleft into the presynaptic neurons, thus modulating the monoaminergic neurotransmission. MATs are targeted not only by their physiological substrates, but also by therapeutic and addictive drugs. Cocaine binds to all MATs and inhibits the uptake of monoamines. Several other psychoactive drugs, e.g. MDMA, enter the presynaptic neuron through MATs or the cell membrane and induce efflux of monoamines, thereby enhancing monoaminergic neurotransmission. By contrast, the antidepressant citalopram exclusively binds to SERT, whereas methylphenidate, the drug for treatment of attention deficit hyperactivity disorder (ADHD), specifically binds to DAT. Controversy regarding the number and function of ligand binding sites in neurotransmitter-sodium symporters arose from conflicting data in crystal structures and molecular pharmacology. We designed novel molecular tools for AFM (atomic force microscopy) that directly measure interaction forces with MATs on the single molecule level. Two distinct force populations of characteristic binding strengths of citalopram to SERT were revealed in the presence of Na⁺. In contrast, in Li⁺-containing buffer, SERT showed only low force interactions. Conversely, the vestibular mutant SERT-G402H merely displayed the high force population. These observations provide physical evidence for the existence of two binding sites in SERT, i.e. a central (S1) site and a vestibular (S2) site, when accessed in a physiological context. Competition experiments revealed that these two sites were allosterically coupled and exerted reciprocal modulation¹. Interaction forces between the cocaine analogue, MFZ2-12, and DAT revealed that two populations of binding strength were pronounced in the presence of Zn²⁺ (10 μM), accompanied by an elevated binding activity. These findings are in accordance with a Zn²⁺ induced outward facing conformational change. Absence of Na⁺, Zn²⁺, or mutation at S422A dramatically reduced the population of strong interaction forces, indicating that it originated from the central S1 binding site. Two populations of unbinding forces were also observed with AFM tips conjugated with dopamine. Substitution of Na⁺ in the buffer with K⁺ or NMDG⁺ disrupted the stronger binding site, whereas acetylation of the lysine residues K92 and K384 in the extracellular vestibule diminished the population of weaker unbinding events. Thus, single molecule force spectroscopy, which allows for the extraction of dynamic information of transmembrane transporters in the native cellular membrane under physiological conditions, provides clear physical evidence of two distinct binding sites in SERT and DAT.

1. Zhu, R. *et al.*, *Angewandte Chemie International Edition* **55**, 1719-1722 (2016).

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