Drug Design

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Snapshot of Antidepressants at Work: The Structure of Neurotransmitter Transporter Proteins

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The protein family of neurotransmitter transporters is the treasure trove of psychopharmacology,[1] yet the way in which they interact with drugs has remained controversial.[2] Now, in two complementary studies, Gouaux and colleagues clarify the molecular binding mode of commonly prescribed antidepressants, explaining the superb druggability of neurotransmitter transporters and setting the stage for the structure-based discovery of improved drugs for psychiatric diseases.[3,4]

Neurotransmitter sodium symporters (NSSs) are sodium-coupled transporters that terminate neurotransmission by removing neurotransmitters from the synaptic cleft.[1] These transporters are implicated in many psychiatric diseases and are the targets of important drugs like tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) which have transformed clinical practice. However, at the molecular level, their mechanism of action has remained poorly understood. One of the reasons was the difficulty in crystallizing eukaryotic transporters, which are integral membrane proteins. The first breakthrough in this field was achieved by Gouaux and co-workers in 2005 when they crystallized LeuT, a remote homologue of eukaryotic NSS, thereby defining a novel protein fold and the overall architecture that is common to all NSSs.[5] LeuT, a bacterial leucine transporter from *Aquifex aeolicus*, consists of 12 transmembrane helices and shares 20–25% sequence identity with human neurotransmitter transporters. The LeuT structure revealed the primary substrate binding site in the protein core and two sodium ions that are thought to provide the driving force of transport against a concentration gradient. In 2007, several groups reported crystal structures of LeuT in complex with several antidepressants, which were bound in an allosteric pocket remote from the substrate binding site.[6] However, this *exo* site in the extracellular vestibule was at odds with many biochemical studies on eukaryotic neurotransmitter transporters, suggesting important differences between prokaryotic and eukaryotic transporters.[2]

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The Gouaux group addressed this discrepancy between bacterial and eukaryotic transporters by two complementary approaches. Wang et al. modified the binding site of the bacterial LeuT transporter by replacing selected active site residues with the corresponding amino acids found in eukaryotic transporters.[4] Indeed, the obtained bacterial–human hybrid transporter mutant LeuBAT had an eukaryotic-like pharmacological profile and bound different SSRIs, SNRIs, and TCAs with highly improved affinity. The authors then solved a panel of cocrystal structures of the LeuBAT mutant with these SSRIs, SNRIs, and TCAs. These drugs all trapped the LeuBAT mutant in an outward-facing open conformation and bound to a position previously identified as the primary substrate binding site for this conformation.[5]

In a second approach Penmatsa et al. solved the cocrystal structure of a bona fide eukaryotic neurotransmitter transporter, the dopamine transporter (DAT) from the fly *Drosophila melanogaster*.[3] Like all other eukaryotic neurotransmitter transporters, the wild-type version of *Drosophila* DAT is highly unstable in purified and solubilized form and not suitable for crystallographic structure determination. Penmatsa et al. solved this problem by deleting flexible loops and termini of the protein, introducing thermostabilizing mutations, and complexing it with an antibody fragment. These techniques have revolutionized the crystallization of integral membrane proteins in the past years, most notably for G protein coupled receptors.[7] The authors then solved the complex of *Drosophila* DAT with the antidepressant nortriptyline, which again bound at the putative substrate binding site in the outward-facing open conformation (Figure 1).

Collectively, these data make a compelling case for the primary binding site of antidepressants. By comparing the different structures, the authors could identify three subsites of the primary binding pocket and the amino acids involved in the interactions with the drugs, thereby providing a generalized structural framework for the binding mode of antidepressants. Their work illuminates how neurotransmitter transporters can accommodate fairly diverse ligands in a deeply buried pocket. This provides a structural explanation for the established extraordinarily high druggability of this protein class, in other words, the comparatively high likelihood to identify suitable ligands.

In one case, Wang et al. also obtained a cocrystal structure of the LeuBAT mutant in complex with two desvenlafaxine molecules, one binding to the putative substrate binding site

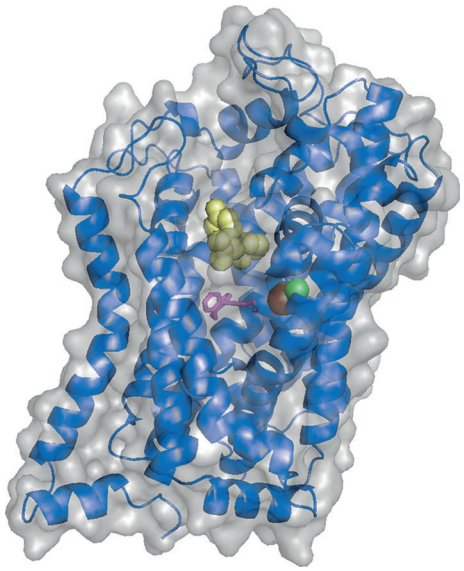

Figure 1. Crystal structure of the thermostabilized dopamine transporter (DAT) of *D. melanogaster* in complex with the antidepressant nortriptyline (pink rods, pdb 4M48). The surface of mdDAT is indicated in gray and its backbone is shown as dark blue ribbons. Part of helix 6 has been removed for clarity. Two bound sodium ions and a chloride ion are shown as brown and green spheres, respectively. The secondary binding site for antidepressants in the extracellular vestibule is indicated in yellow.

and the other to the extracellular vestibule. This secondary binding site corresponds to the *exo* site previously observed for LeuT[6] and might represent the low-affinity allosteric pocket previously postulated for antidepressants like escitalopram.[8a] Whether drugs can be designed specifically for this secondary binding site and whether these compounds would have altered pharmacological properties remains to be elucidated.[8b]

A minor shortcoming of the present structures is that the protein constructs used are inactive for neurotransmitter transport and that they reflect a mixed dopamine/norepinephrine/serotonin transporter pharmacology. Thus, they cannot explain the origin of transporter selectivity, for example, of SSRIs for the human serotonin transporter. Nonetheless, the findings by Gouaux and colleagues are landmark studies for the future development of neurotransmitter transporter ligands. In this respect, the biggest implication might not be for improving activity for the human monoamine transporters as such, since the existing compounds already have reached a high level of optimization. However, other promising targets within the NSS family like the transporters for glycine (GlyT), GABA (GAT), and neutral amino acids (e.g. SLC6A15), which also have been implicated in psychiatric disorders, will likely benefit substantially from the data and methods provided by the Gouaux group. Furthermore, an important contemporary trend in the discovery of improved psychopharmacological drugs is the combination of activities for multiple targets in one molecule,[9] as exemplified by the recently approved antidepressant Vortioxetine (LuAA21004).[10] Towards this goal, the findings by Wang et al. and by Penmatsa et al. provide a basis for rational structure-based design in polypharmacology, that is, the design of compounds that include the inhibition of monoamine transporters as a key pharmacological component.

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