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## Getting a Better Handle on Antidepressant Action

Researchers have identified a new mechanism by which tricyclic antidepressants (TCAs) inhibit neurotransmitter transporters—a discovery that may improve the design of new antidepressants that are more effective than the TCAs currently on the market. TCAs, which have been prescribed for decades, have been largely supplanted by selective serotonin reuptake inhibitors because of their lack of specificity.

Howard Hughes Medical Institute investigator Eric Gouaux at Oregon Health & Science University (OHSU) and colleagues Satinder K. Singh and Atsuko Yamashita published their findings August 8, 2007, in an advance online publication in the journal *Nature*. Singh, the paper's first author, was an HHMI predoctoral fellow and is now a postdoctoral fellow at OHSU. Yamashita, formerly with the Gouaux group at Columbia University, is now a team leader at the RIKEN SPring-8 Center in Japan.

The researchers began their studies with the goal of understanding how TCAs interact with their clinical target, sodium-coupled neurotransmitter transporters. These transporters mop up neurotransmitters from the synapse, the junction between neurons. Neurotransmitters are molecules that neurons use to communicate with neighboring neurons. TCAs work by inhibiting the reuptake of neurotransmitters by neurons.

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Disorders such as depression, epilepsy, autism, or obsessive-compulsive disorder can result from impaired function of sodium-coupled neurotransmitter transporters. Thus, these molecules are the target of a variety of drugs, including TCAs.

It has been a great challenge, however, to understand precisely how these molecules function and interact with drugs. The problem, Gouaux said, is that the transporters found in humans are not amenable to study.

“It’s tough to get cells to make much transporter, and once you isolate the protein, it just falls apart, like a soufflé that falls coming out of the oven,” he said. So the researchers focused on a sturdier transporter in bacteria that functions similarly. That molecule, called LeuT, is used by the bacterium *Aquifex aeolicus*, which thrives in superheated deep-sea vents. LeuT’s job is to transport the amino acid leucine across the bacterial membrane.

Singh explored how the TCA clomipramine, which she had found to be a potent inhibitor of LeuT, attached to the bacterial transporter. Singh used x-ray crystallography to develop a detailed structure of clomipramine attached to LeuT. She also analyzed how two other TCAs, desipramine and imipramine, attached to LeuT. In x-ray crystallography studies, researchers bombard protein crystals with x-rays and then deduce the structure of the protein by analyzing the diffraction pattern produced by the x-rays.

One plausible way for a drug to interfere with a transporter’s function is by physically blocking the part of the molecule that binds to the molecule to be transported. Singh’s steady-state kinetic data and crystallographic studies revealed, however, that clomipramine and the other TCAs do not attach to the same site on the transporter as leucine. Rather, they plug into a cleft in a different part of the transporter and lock it into a conformation that traps leucine, preventing it both from passing through and being released. This cleft is on the region of the transporter that juts outside the cell.

Similar findings were reported in a research article published in the August 9, 2007, issue of *Science*, by Da-Neng Wang, Maarten Reith and colleagues at New York University. They found that the TCA desipramine binds LeuT in the same cleft. The two groups diverge in their conclusions about the relevance of the TCA site in LeuT to the antidepressant site in the human neurotransmitter transporters, however. Wang, Reith, and colleagues argue that it is identical, whereas Singh and her colleagues are more cautious, suggesting that the TCA site is different and probably located ‘deeper’ into the human transporter, close to or overlapping with the substrate site.

Nevertheless, the concept that a molecule binding to an allosteric site may inhibit a transporter by stabilizing an occluded or locked state is one that had not been described previously. Singh, Gouaux, and Atsuko hypothesized that the TCA molecules slowed the release of leucine from the transporter. Sure enough, in her experiments, Singh found that the transporter released leucine

about 700 times slower when clomipramine was attached. “When I followed the reaction, the off-rate was so slow with clomipramine that it took one day. But with no inhibitor, the leucine fell off in less than ten minutes,” she said.

“This study defines a new principle for inhibition of this class of sodium-coupled transporters,” said Gouaux, “and that is the stabilization of this so-called occluded state. The dogma in this field has been that molecules had to be designed to competitively inhibit the transport process by acting as a surrogate yet non-transported substrate. But nobody had any evidence to suggest that stabilization of this occluded state was the mechanism.”

Gouaux said that discovery of the new mechanism may spur development of improved inhibitors. “There is no question that knowledge of the mechanistic principles and details of this drug-binding site will be important in developing a new class of molecules that act very specifically on only a selected type of transporter,” he said. “You can certainly come up with molecules that potently inhibit in this manner, and they don't need to look like the substrate. They can look very different and still plug this extracellular vestibule.

In future studies, Gouaux said he and colleagues will seek to isolate and crystallize the delicate human transporter for structural studies to establish if the basic same inhibitory mechanism is at work.