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Structure/Function Relationships in Serotonin Transporter: New Insights from the Structure of a Bacterial Transporter

G. Rudnick

Department of Pharmacology, Yale University School of Medicine,
New Haven CT, 06520-8066, USA
gary.rudnick@yale.edu

Abstract Serotonin transporter (SERT) serves the important function of taking up serotonin (5-HT) released during serotonergic neurotransmission. It is the target for important therapeutic drugs and psychostimulants. SERT catalyzes the influx of 5-HT together with Na^+ and Cl^- in a 1:1:1 stoichiometry. In the same catalytic cycle, there is coupled efflux of one K^+ ion. SERT is one member of a large family of amino acid and amine transporters that is believed to utilize similar mechanisms of transport. A bacterial member of this family was recently crystallized, revealing the structural basis of these transporters. In light of the new structure, previous results with SERT have been re-interpreted, providing new insight into the substrate binding site, the permeation pathway, and the conformational changes that occur during the transport cycle.

Keywords Serotonin · Transporter · Structure · Mechanism · Permeation

1 General Background and Significance of SERT

The neurotransmitter transporters are plasma membrane proteins that take up extracellular neurotransmitters after release and thereby terminate the transmitters' action at extracellular receptor sites. These plasma membrane neurotransmitter transporters represent the first step in the process of trans-

mitter recycling. Subsequent sequestration by synaptic vesicles requires a second transport system in the vesicular membrane. Although the structure and mechanism of the vesicular neurotransmitter transporters are distinct from those of the plasma membrane, the two systems work together to transport extracellular neurotransmitters into the synaptic vesicle, where they are available for release by exocytosis (Rudnick 2002).

The plasma membrane neurotransmitter transporters use transmembrane ion gradients of Na^+, Cl^-, and K^+ and an internal negative membrane potential for transport of their substrate neurotransmitters (Rudnick and Clark 1993; Rudnick 2002). Transporters responsible for reuptake of neurotransmitters across the plasma membrane of neurons and glia fall into two gene families (Amara 1992). Most small neurotransmitters, including glycine, γ-aminobutyric acid (GABA), dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT), are transported by proteins belonging to the family designated the neurotransmitter sodium symporter (NSS) family 2.A.22 by Saier (1999). Glutamate, however, is transported by a family of mono- and dicarboxylic amino acid transporters, the DAACS family (Saier 1999). Proteins in both families play important roles in brain function.

Serotonin transporter (SERT) is a member of the NSS family that selectively transports 5-HT into nerve cells together with Na^+ and Cl^- and, in the same reaction, transports a K^+ ion out of the cell. SERT is inhibited by a variety of compounds that are used to treat clinical depression, including fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), and citalopram (Celexa). These compounds were synthesized as selective serotonin reuptake inhibitors (SSRIs) based on the observation that compounds useful as antidepressants, such as imipramine, inhibited serotonin transport. The widespread use of serotonin reuptake inhibitors makes SERT a molecule of high clinical interest.

In addition to drugs that specifically target SERT, this transporter is also affected by cocaine and amphetamines—psychostimulant drugs that are widely abused. Cocaine acts as a simple inhibitor of SERT and the closely related NSS transporters for NE and DA, NET and DAT, respectively (Gu et al. 1994). Amphetamine and its congeners, however, have a more complex mechanism of action. These compounds are substrates for SERT, NET, and DAT but also diffuse into cells because of their high membrane permeability. This ability to cross membranes in their unprotonated, neutral form allows amphetamines to dissipate the internally acid pH difference (ΔpH) across the synaptic vesicle membrane (Schuldiner et al. 1993). Because this ΔpH is used as an important driving force for accumulation of 5-HT, NE, and DA by synaptic vesicles, collapsing the ΔpH causes release of accumulated neurotransmitter into the cytoplasm. Transport of amphetamines by SERT, NET, or DAT leads to accumulation of Na^+ in the cytoplasm (Khoshbouei et al. 2003), and the combination of high cytoplasmic neurotransmitter and increased cytoplasmic Na^+ leads to reversal of the plasma membrane transporter and appearance of neurotransmitter outside the cell (Rudnick 2002). Thus, both cocaine and amphetamines

lead to more neurotransmitter in the synapse, but in the case of amphetamine, cellular stores are actively released, while cocaine raises synaptic transmitter by blocking re-uptake. Among the variety of amphetamine derivatives, MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") is more selective toward releasing 5-HT from serotonergic neurons, and this selectivity is due to MDMA's higher affinity for SERT relative to its affinity for NET or DAT (Wall et al. 1995).

2 Mechanism of Transport

SERT, like other transporters, is believed to function by alternately exposing a substrate binding site to the cytoplasmic and extracellular faces of the plasma membrane. To understand such a mechanism in detail requires knowledge of four key properties of the protein: (1) The nature of the binding site determines how the transporter can selectively transport one substrate and not another. In cases, like SERT, where ions are cotransported with substrate, the relative positioning of substrate and ion binding sites may be critical for coupling. (2) The pathways that the substrate and ions pass through from one side of the membrane to the binding site and then from the binding site to the other side of the membrane need to be tightly coupled to each other so that they are not both open simultaneously, which would lead to uncoupled flux through the transporter. (3) The transporter must undergo conformational changes that close access from one side of the membrane and open access to the other. (4) Occupancy of the binding sites must control conformational changes so that they occur only when the appropriate ligands are bound. Otherwise, the transporter would catalyze uncoupled flux of any solute that occupied its binding site.

According to a mechanism proposed to describe 5-HT transport, SERT binds Na^+, Cl^-, and 5-HT^+ in a 1:1:1 stoichiometry and only then undergoes a conformational change that occludes the binding site from the extracellular medium and exposes it to the cytoplasm (Nelson and Rudnick 1979). After dissociation of Na^+, Cl^-, and 5-HT^+, the transporter returns to its original conformation only after binding a cytoplasmic K^+ ion and releasing it to the extracellular medium. The overall stoichiometry of this process is a 1:1:1:1 electroneutral exchange of K^+ with Na^+, Cl^-, and 5-HT^+ (Rudnick and Nelson 1978; Talvenheimo et al. 1983; Rudnick 1998).

Evidence for this mechanism originally came from studies using platelet plasma membrane vesicles (Rudnick 1977). These studies provided evidence for the stoichiometry and supported the movement of K^+ in a step distinct from the one in which 5-HT was transported (Nelson and Rudnick 1979). SERT is also capable of conducting ionic current that is induced by 5-HT (Mager et al. 1994; Lin et al. 1996; Cao et al. 1997; Cao et al. 1998). Although this would, on

the surface, appear to argue against a coupled electroneutral stoichiometry, it has become clear that SERT catalyzes an uncoupled flux in addition to the coupled transport process. An alternative mechanism has been put forward in which 5-HT and Na^+ movement are coupled within a channel (Petersen and DeFelice 1999; Adams and DeFelice 2002), but this mechanism does not explain how K^+ countertransport could be coupled to 5-HT uptake in an electroneutral process. A recent study demonstrated that interaction with syntaxin 1a could block the uncoupled current, revealing the coupled electroneutral process with the same stoichiometry that was originally proposed (Quick 2003).

For electroneutral 5-HT transport coupled to Na^+, Cl^-, and K^+, the conformational changes that allow these solutes to cross the membrane must serve two functions. First, they must open up the binding site alternately to each side of the membrane to allow binding and dissociation of solutes. Second, they must prevent uncoupled movement of solutes. For example, if the transporter were constantly interconverting between cytoplasmic- and extracellular-facing forms regardless of what solutes were bound, it would catalyze only downhill leakage of 5-HT, Na^+, K^+, and Cl^-. For any transporter to be stoichiometrically coupled, its conformational changes must be linked to the occupancy of the binding site. For SERT, the conformational change should occur when 5-HT, Na^+, and Cl^- are bound or when K^+ is bound, but not when the binding site is only partly occupied (see Fig. 1).

A major advance in this process resulted from the discovery that the genomes of many prokaryotes (bacteria and archaea) contained genes coding for proteins quite homologous to neurotransmitter transporters. In 2003, the first evidence became available showing that these proteins were actually transporters (Androutsellis-Theotokis et al. 2003). It showed that the TnaT protein of *Symbiobacterium thermophilum* was a Na^+-dependent tryptophan transporter with properties similar to those of other NSS transporters. Recently, the laboratory of Eric Gouaux provided a high-resolution structure from another bacterial homolog, LeuT from *Aquifex aeolicus* (Yamashita et al. 2005). Although this structure will certainly differ in details from the structure of the mammalian proteins, it provides a framework for designing further experiments toward a variety of goals, among which are to test the relevance of the structure, to define the particular differences between the bacterial and mammalian transporters, and to understand the molecular motions within the structure that lead to transport.

The structure of the *A. aeolicus* leucine transporter ($LeuT_{Aa}$) provides some surprises, some unique features, and many opportunities to explore mechanistic issues relevant to neurotransmitter transport. An unusual aspect to this structure is that it contains a repeat of two groups of five transmembrane domains in opposite topological orientations. Because of the high resolution of the structure, the Gouaux group was able to identify two Na^+ ions bound together with leucine at the active site, thus providing a structural basis for coupling of Na^+ and solute fluxes.

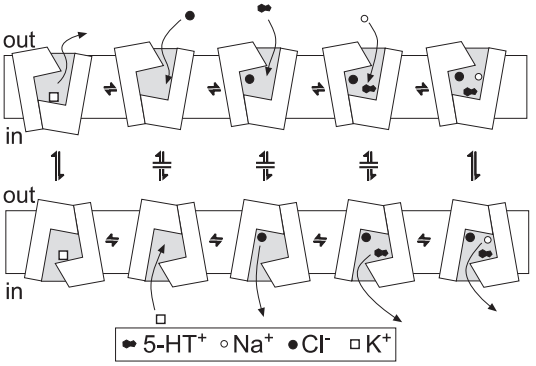

Fig. 1 Possible mechanism of serotonin transport. Transport of 5-HT together with Na^+ and Cl^- ions requires binding of each solute to the transporter. These binding events are depicted in the three steps on the *upper right* of the figure. There is no evidence that the binding sequence is strictly ordered. Only after all three solutes are bound is the transporter able to undergo a series of conformational changes that closes off access to the extracellular medium and exposes the binding site to the cytoplasm. This conformational change is depicted on the *right side* of the figure. After dissociation of 5-HT, Na^+, and Cl^- on the cytoplasmic side of the plasma membrane, as shown by the three *rightmost* steps on the *lower* part of the figure, a cytoplasmic K^+ ion is able to bind (*lower left*). Once K^+ has bound, SERT is able to undergo another series of conformational changes that closes off access to the cytoplasm and exposes the binding site to the extracellular medium. Dissociation of K^+ to the medium completes the cycle. Note that for effective coupling of 5-HT influx to both influx of Na^+ and Cl^- and efflux of K^+, transitions between the extracellular-facing and cytoplasmic-facing forms of SERT should occur only when the binding site is occupied with 5-HT, Na^+, and Cl^- or with K^+

3 Topology

The primary sequence of most NSS family members predicts 12 transmembrane (TM) domains connected by hydrophilic loops. However, several prokaryotic sequences predict only 10 TMs. The fact that the central core of LeuT, including the substrate and Na^+ binding sites, is formed by two copies of a 5-TM repeat provides an explanation for the functionality of 10-TM transporters and suggests that TM11 and TM12 are not always required for transport function. In the mammalian transporters, glycosylation sites in the second extracellular loop (EL2, between TM3 and TM4) indicated that EL2 is extracellular (Tate and Blakely 1994). For SERT, many residues predicted by the initial topological predictions to lie in hydrophilic loops were demonstrated to be accessible from the appropriate side of the membrane (Chen et al. 1998; Androutsellis-Theotokis and Rudnick 2002), indicating a 12-TM structure with NH_2- and COOH-termini in the cytoplasm. These studies extensively utilized cysteine-scanning mutagenesis of internal and external loops and transmembrane do-

mains. Figure 2 shows a summary of some of results for SERT superimposed on a topology diagram generated from the crystal structure of LeuT and using an alignment of the NSS family. The highlighted positions, where various studies demonstrated chemical reactivity with hydrophilic reagents, indicate many residues in regions exposed to solvent in the structure (Chen et al. 1997a, b, 1998; Chen and Rudnick 2000; Androutsellis-Theotokis et al. 2001; Ni et al. 2001; Androutsellis-Theotokis and Rudnick 2002; Henry et al. 2003; Mitchell et al. 2004; Sato et al. 2004).

In addition to those positions that are clearly in the hydrophilic extracellular or cytoplasmic domains, cysteines were modified at many positions that the LeuT structure predicts to be inaccessible from either face of the membrane. The observation that residues predicted by the structure to be buried were nonetheless accessible in SERT suggests that other conformations of the transporter expose these residues in other conformations of the protein. Thus, the form of LeuT that crystallized is likely to represent only one very restricted conformation of a transporter that must undergo conformational changes to allow substrates to bind and dissociate from both sides of the membrane. In fact, the binding site in the LeuT structure contains the substrate and two Na^+ ions occluded from both faces of the membrane. Conformational changes are required to allow these solutes into and out of the binding site.

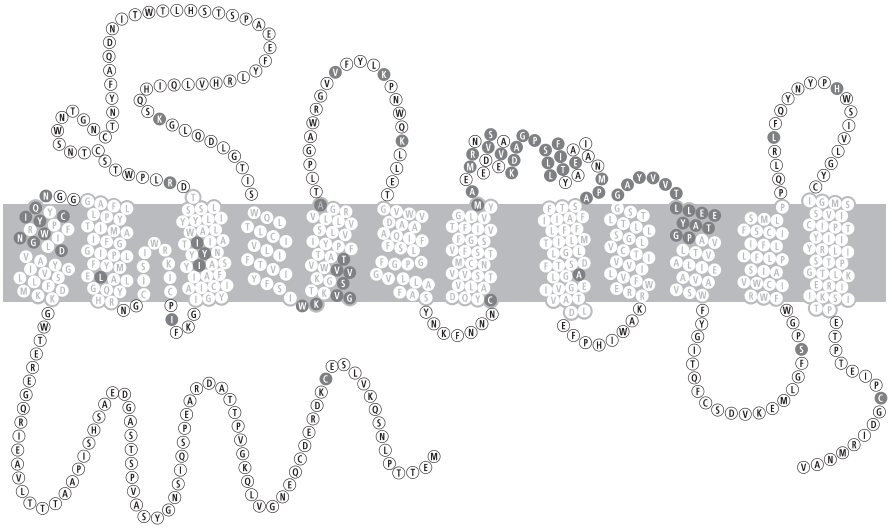

Fig. 2 Topology diagram of SERT. The sequence of SERT was aligned with that of $LeuT_{Aa}$ and presented as a topology diagram, using the beginnings and ends of each helix from the LeuT structure. *Shaded* residues are those where mutation to cysteine or lysine was found to introduce reactivity toward hydrophilic compounds such as MTS reagents. Not all positions were tested, and positions where reactivity was not detected are not marked

4
The Permeation Pathway

Keller et al. (2004) and Zhang et al. (2005) were able to react cysteines in the extracellular half of TM10 and the cytoplasmic half of TM5 that were occluded in the LeuT structure. For example, in the LeuT structure, access of substrate to the extracellular medium is blocked in part by a salt bridge between Arg-30 and Asp-404 (Yamashita et al. 2005). The corresponding residues in SERT are Arg-104 and Glu-493. However, in SERT, access in the EL5 region containing Glu-493 continued up to Pro-499. According to the LeuT model, this position is in TM10, almost in the middle of the membrane (Fig. 3). An example of the same phenomenon from the cytoplasmic side of the membrane is in intracellular loop (IL)2, where positions up to Tyr-289 were found to be accessible to reagents on the cytoplasmic side. In the LeuT structure, Phe-203, which corresponds to SERT Tyr-289 is in TM5 more than halfway across the membrane from IL2 (Fig. 3; Yamashita et al. 2005). It is noteworthy that TM5 and TM10 are related in that each one is the last TM in the two repeated 5-TM structural units that make up the core of the structure. The space between the intracellular end of the exposed region of TM10 and the extracellular end of the exposed region of TM5 contains binding site residues for leucine and Na^+ formed by TMs 1, 3, 6, and 8. Thus, the accessibility of residues in TM5 and TM10 might indicate that these helices form part of the permeation pathway that allows substrates to enter and exit their binding sites.

In the LeuT structure, leucine is occluded, and there is no pathway for it to dissociate either to the periplasmic or cytoplasmic sides of the membrane (Yamashita et al. 2005). Although leucine is occluded in the crystal structure, only a few residues block its exit to the periplasmic (external) side of the membrane. However, there is almost 20 Å of packed protein structure separating leucine

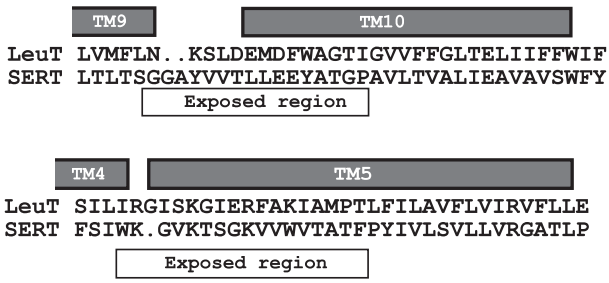

Fig. 3 Exposure of TM domains to aqueous reagents. The sequence of rat SERT was aligned with that of LeuT in the EL5–TM10 region and the IL2–TM5 region. The *shaded* regions indicate the TM helices and the *open boxes* indicate the regions that were accessible to modification with [2-(trimethylammonium)ethyl]methanethiosulfonate (MTSET) (for cysteine replacements in EL5–TM10) or (2-aminoethyl)methanethiosulfonate (MTSEA) (for cysteine replacements in IL2–TM5)

from the cytoplasmic face of the protein. Taken at face value, the pathways from the periplasmic and cytoplasmic faces to the binding site would appear to be quite different. However, another interpretation is that the crystal structure represents an intermediate in which the cytoplasmic pathway is closed and the periplasmic pathway is mostly open. The blockade of the periplasmic permeation pathway (by Tyr-108, Phe-253, Arg-30, and Asp-404) might even be properties of an intermediate that is not on the normal reaction path but that is particularly stable in the crystal. Additional evidence exists that the periplasmic permeation pathway is more condensed in the cytoplasmic-facing form than is suggested by the LeuT structure. Residues in the tip of EL4 (between TMs 7 and 8), Tyr-107, and Ile-108 form parts of the periplasmic pathway in LeuT. In SERT, the corresponding residues are largely protected by 5-HT in the presence of NaCl (Henry et al. 2003; Mitchell et al. 2004), conditions likely to favor the cytoplasmic-facing form. Because the crystal structure may represent a form of LeuT close to the periplasmic-facing form, it is quite possible that—in the cytoplasmic-facing form—the external pathway exists in a more condensed conformation.

5 The Substrate Binding Site

Evidence from many laboratories (Chen et al. 1997b; Barker et al. 1998, 1999; Chen and Rudnick 2000; Adkins et al. 2001; Henry et al. 2003; Melamed and Kanner 2004; Zhou et al. 2004) strongly suggested that the binding site for the substrate and ions is formed, at least in part, by TM1 and TM3. TM2, although it is adjacent to both TM1 and TM3 in the primary sequence, does not contribute directly to the binding site in LeuT, and results from SERT are in agreement that—although mutations in this region have effects on expression and K_M for substrate—most of the positions in TM2 were neither accessible to extracellular reagents nor affected by substrate binding (Sato et al. 2004). However, mutations in TM2 of DAT have strong effects on the affinity of cocaine (Chen et al. 2005; Sen et al. 2005) and may represent part of an inhibitor binding site or it might contribute to the position of TMs 1 and 6. In addition, the LeuT structure revealed that at least one residue, Leu-137—thought to be part of the IL1 loop between TM2 and TM3—is actually part of TM2. L137C reacted with (2-aminoethyl)methanethiosulfonate (MTSEA), and the reaction was inhibited by 5-HT and cocaine (Androutsellis-Theotokis and Rudnick 2002). Similarly, Ala-441 was previously thought to be part of IL4 between TM8 and TM9. The LeuT structure revealed that this position is in TM8 only one helical turn away from binding site residues. When expressed in intact cells, A441C did not react with MTSEA, but in membrane preparations MTSEA inactivated A441C binding activity and this reaction was inhibited by 5-HT and cocaine.

We previously had no indication that other TM domains contributed to the permeation pathway, although analysis of channel proteins strongly suggested

that it must consist of more than just TM1 and TM3 (Spencer and Rees 2002). From the LeuT structure, it is apparent that the two corresponding TMs from the second 5-TM repeat, TM6 and TM8, also contribute to the binding site. It will be important to examine residues in TM6 and TM8 to evaluate their contribution to the SERT binding site for 5-HT, Na^+, and drugs such as cocaine, amphetamines, and antidepressants.

The structure of LeuT contains two bound Na^+ ions in proximity to the bound leucine. The residues responsible for coordination of Na^+ are not identical in SERT. The most pronounced difference is that, in LeuT, the leucine carboxyl group coordinates one of the Na^+ ions (Na1). In SERT, as in NET and DAT, the substrate contains no carboxylate group that can fulfill this function, but there is an aspartate residue at position 98 in SERT (Gly-24 in LeuT) that is positioned to take its place. Mutation of this aspartate to glutamate or cysteine was strongly inhibitory for SERT function (Barker et al. 1999; Henry et al. 2003). A second bound Na^+ ion in the LeuT structure (Na2) is coordinated by five residues in the LeuT structure. Three of these residues are identical in SERT. One of the two non-identical residues coordinates with Na2 through its carbonyl oxygen, and the other is a Thr-354 in LeuT and an Asp-437 in SERT. Because Asp-437 could participate in coordinating a Na^+ ion in SERT similar to Na2 in LeuT, it is possible that SERT also binds 2 Na^+ ions. Indeed, previous results comparing the Na^+ dependence of 5-HT transport and imipramine binding by SERT suggested that 2 Na^+ ions were involved in the latter process (Talvenheimo et al. 1983).

However, this poses questions about how Na1 and Na2 are related to Na^+ ions cotransported with substrate. Some transporters in the NSS family, such as GAT-1 and GlyT1b, are known to transport 2 Na^+ ions with each substrate molecule (Keynan and Kanner 1988; Roux and Supplisson 2000). SERT Na^+ stoichiometry has been determined by two methods and found to be 1 Na^+ per 5-HT (Talvenheimo et al. 1983; Quick 2003). The Na^+ binding stoichiometry of LeuT is 2, but the Na^+ stoichiometry for transport is unknown. Many interesting mechanistic questions remain to be answered regarding ion coupling in SERT. Is binding stoichiometry always the same as transport stoichiometry, or is it possible that only 1 of the 2 Na^+ ions in the structure is transported? Similarly, does the single sodium symported with 5-HT by SERT represent a single bound Na^+ ion, or is an additional, non-transported Na^+ bound to SERT? Does either of the Na^+ sites predicted in SERT by the LeuT structure represent the site used for K^+ antiport? How is Cl^- cotransport coupled to 5-HT in SERT?

6 Conformational Changes

Many of the positions indicated by Fig. 2 to react with MTS (methanethiosulfonate) reagents are not close to the binding site for substrate and ions as

defined by comparison with the LeuT structure. And yet, for many of these positions, reactivity is sensitive to the presence of 5-HT or cocaine. Because of their distance from the substrate binding site, it is unlikely that these residues are directly occluded by 5-HT or ion binding, but the changes in reactivity are likely to reflect conformational changes in response to occupation of the binding site. Furthermore, many of the changes that were observed required not just 5-HT, but Na^+ and Cl^- as well, suggesting that the change in accessibility represented entry of SERT into the transport cycle.

As discussed in the previous section, two residues in TM3 were identified as being close to the substrate binding site. Part of the evidence for this conclusion was the observation that 5-HT could protect a cysteine at those positions from modification by MTS reagents (Chen et al. 1997b). Further studies demonstrated that 5-HT protected even in the absence of Na^+, indicating that 5-HT binding was not Na^+-dependent (Chen and Rudnick 2000). Thus, it was important to discover that in many SERT cysteine mutants, the effect of 5-HT on reactivity of a cysteine residue required Na^+ (Androutsellis-Theotokis et al. 2001) or both Na^+ and Cl^+ (Mitchell et al. 2004; Sato et al. 2004).

To put these various ion requirements in perspective, it is helpful to consider the likely transport pathway for SERT, as deduced from a variety of approaches (Rudnick 2002). In this pathway, Na^+, Cl^-, and 5-HT all bind from the extracellular side of the membrane to form a quaternary complex with the transporter (see Fig. 1). Only when this complex is formed will the transporter undergo a conformational change to expose the 5-HT binding site to the cytoplasm. After dissociation of Na^+, Cl^-, and 5-HT to the cytoplasm, the binding site can accept a K^+ ion that allows a second conformational change, returning SERT to its original extracellular-facing conformation. After K^+ dissociation to the extracellular medium, the cycle can start over. A key feature of this process is the requirement for a particular set of solutes (either Na^+, Cl^-, and 5-HT or K^+) to be bound before the protein can change conformation. This requirement is responsible for the stoichiometric coupling of 5-HT, Na^+, Cl^-, and K^+ (Rudnick 1998).

Because conformational changes that affect the accessibility of the 5-HT and ion binding sites are such a critical part of the mechanism, it is important to define each of the potential conformations. We assume that there is a form of SERT in which the 5-HT and ion binding sites are directly accessible from the extracellular medium. This conformation is represented by the upper part of Fig. 1 and will be referred to as the "extracellular-facing form." Another conformation (the lower part of Fig. 1) must release 5-HT and ions from their binding sites to the cytoplasm and this will be referred to as the "cytoplasmic-facing form." There may be intermediate forms, such as the form of LeuT in the crystal structure, in which the binding sites are exposed to neither side. These forms will be referred to as "occluded." The pathways from the binding site to the extracellular medium or the cytoplasm that are properties of the extracellular-

facing and cytoplasmic-facing forms, respectively, will be referred to as the extracellular and cytoplasmic permeation pathways, respectively.

It would be useful to assign the various changes in cysteine accessibility with different states of the transporter, so as to understand which parts of the protein participate in the conformational changes accompanying binding and transport reactions. It is possible to assign some changes with binding and others with the transport steps. For example, cysteines at some positions (such as I172C and Y176C) were protected by 5-HT or inhibitor binding, did not depend on Na^+, and were protected both at 25 °C and 4 °C (Chen and Rudnick 2000). This can be interpreted as a simple steric occlusion of the reactive residue, which is a property of the outward-facing transporter and does not require conformational changes.

At other positions (such as Cys-357), both 5-HT and inhibitors protected against cysteine modification, but the protection required Na^+ and was observed at 25 °C but not 4 °C (Androutsellis-Theotokis et al. 2001). This behavior suggests an allosteric effect due to a conformational change that occurs when both 5-HT and Na^+ are bound to SERT. It probably does not represent the translocation step, because the protection was seen with both 5-HT and the non-transported inhibitor cocaine. Apparently, binding induced a conformational change that precedes the translocation event.

A third set of residues (such as S404C and Y107C and I108C) were protected only by 5-HT and not cocaine, and the protection required both Na^+ and Cl^- (Henry et al. 2003; Mitchell et al. 2004). This behavior probably represents the conformational change that actually translocates 5-HT across the membrane. The reasoning is as follows: (1) The effect is allosteric, suggesting a conformational change, since some residues were protected (Mitchell et al. 2004) while others were potentiated (they reacted faster with MTS reagents when 5-HT, Na^+, and Cl^- were present) (Sato et al. 2004). (2) Only substrates but not non-transported inhibitors such as cocaine promote the change in reactivity (Mitchell et al. 2004; Sato et al. 2004). (3) Other SERT substrates, such as MDMA, could replace 5-HT (Sato et al. 2004). These data suggest that the presence of 5-HT, Na^+, and Cl^- transforms SERT from a predominantly extracellular-facing conformation to one that is predominantly cytoplasmic-facing, having transported 5-HT and released it on the cytoplasmic side of the membrane.

The ability to manipulate the state of SERT and to determine the effects on accessibility of cysteine residues placed at specific positions will allow the testing of possible transport mechanisms. The goal is to understand how the conformational changes within SERT lead to alternate accessibility of the binding site from the two sides of the membrane. To accomplish this goal, it will be necessary to use biochemical approaches with the functional protein in its native environment. The structure of LeuT provides a framework for these studies, but it does not provide much information about dynamic changes in the transporter structure.

7 Future Directions

Many aspects of SERT structure remain unresolved despite the major advance in our understanding provided by the structure of LeuT. As described above, the difference in ion coupling between SERT and LeuT must be reflected in differences in the structure of the substrate binding site. In addition, there are three regions of SERT where the structure of LeuT provides little or no information. The first of these is EL2, which is much longer in SERT than in LeuT. EL2 is likely to be important for functional expression of SERT because replacing part or all of it with its corresponding sequence from NET led to a protein inactive for transport (Stephan et al. 1997; Smicun et al. 1999). The additional sequence not present in LeuT includes glycosylation sites (Tate and Blakely 1994) and a highly conserved pair of cysteine residues likely to form a disulfide (Chen et al. 1997a). Mutations in these cysteines, or modification of one when the other was mutated, led to severe loss in activity (Chen et al. 1997a). Thus, it is likely that the parts of EL2 that are unique to animal members of the NSS family are functionally important in ways that are not addressed by the LeuT structure.

SERT, like most neurotransmitter transporters, contains much longer NH_2- and COOH-terminal regions than does LeuT. The N-terminal region of SERT has been implicated in the regulation of ion conductance by syntaxin 1a (Quick 2003). It is likely that these domains are important for regulation through interactions with other intracellular pathways. The NH_2- and COOH-terminal regions are also likely targets for agents that control the subcellular localization of SERT, as has been demonstrated for the related norepinephrine and GABA transporters (Perego et al. 1997; Muth et al. 1998; Gu et al. 2001; Farhan et al. 2004). The structure of these domains and their potential interaction with the intracellular face of the central region of SERT are still unknown, and will doubtless be the subject of future study.

Because they show a static structure, the images of LeuT cannot tell us how transporters in this family move substrate and ions from the cell exterior to the binding site and then to the cytoplasm. These movements require conformational changes involving the transmembrane domains and possibly also the hydrophilic loops that connect them. Moreover, these movements are triggered by the binding of appropriate substrates and ions to the transporter (in the case of SERT, Na^+, Cl^-, and 5-HT for the forward reaction and K^+ for the return). Understanding the mechanism by which these binding events allow and control the conformational changes, and comprehending the nature of the conformational changes themselves, are important goals for future research in this area.

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