

Preface

Neurotransmission is a multicomponent process. Transmitters, released by neuronal activity, act on pre- and postsynaptic receptors, and many books detail advances in the receptor field. In addition, after their release from nerve endings, transmitters are removed from the neuronal vicinity by uptake into neuronal or glial cells by specific transporter proteins that have been studied intensely over the last 30 years; this information is scattered throughout numerous publishing vehicles. Therefore, the primary aim of this second edition of *Neurotransmitter Transporters: Structure, Function, and Regulation* is to offer a comprehensive picture of the characterization of neurotransmitter transporters and their biological roles. The transporter field has moved forward in stages. In the first phase, progress came from the use of substrate or blocker ligands selectively targeting transporters, the application of model systems allowing the study of transmitter transport shielded from storage, and the development of mathematical models for describing transport phenomena. In the second phase, roughly covering the last decade, advances in DNA techniques allowed the cloning of numerous genes coding for different transporter proteins. In the current, third stage, a wealth of information is being accumulated in studies relating transporter structure with function, experiments addressing regulation by posttranslational transformation, investigations into transport modulation by trafficking processes and genomic influences, characterization of channel properties of transporters by electrophysiological approaches, and the creation of transgenic animals under- or overexpressing a given transporter protein.

The first edition of *Neurotransmitter Transporters: Structure, Function, and Regulation* was published at the brink of the above 2nd and 3rd phase; the current, second edition finds the transporter field fully in the 3rd phase. This second edition of *Neurotransmitter Transporters: Structure, Function, and Regulation* has been put together with the goal of retaining most of the original material in describing for each transporter the cloning history as well as including new progress in characterizing its structure, function, regulation, and physiological relevance.

Interest in neurotransmitter transporters has increased tremendously, along with clearer understanding of their roles in the pathophysiology of schizophrenia, Tourette's syndrome, Parkinson's disease, affective disorders, attention deficit/hyperactivity disorder, neurotoxin accumulation and removal, brain ischemia, amyotrophic lateral sclerosis, or in the mechanism of action of drugs of abuse, antidepressants, and antiepileptics. Thus, *Neurotransmitter Transporters: Structure, Function, and Regulation, Second Edition* will be of interest to scientists, graduate students, and advanced undergraduates who seek a comprehensive overview of this active field in neuroscience. Selected chapters will also be of interest to physicians who are carrying out imaging and postmortem measurements of neurotransmitter transporters in the human brain, or physicians who study gene linkages and polymorphisms in relation to psychiatric and other complex diseases.

Each chapter of *Neurotransmitter Transporters: Structure, Function, and Regulation* offers a critical summary and synthesis of the progress in characterizing each transporter in terms of structure–function relationships and regulation. Chapters 1–9 focus on various neurotransmitter transporters located in neuronal or glial plasma membranes and in synaptic vesicles. The Na⁺, Cl⁻-dependent plasma membrane transporters are described for monoamines (Chapters 1–5) and for a number of compounds including amino acids (GABA, glycine, proline, betaine, taurine, and creatine (Chapters 6 and 7). The separate family of Na⁺-dependent glutamate transporters is discussed (Chapters 7 and 8) as well as the family of vesicular transporters for monoamines, acetylcholine, and GABA/glycine (Chapter 9). Chapters 10–13 cover a variety of issues relevant to transporter structure and function. Chapter 10 describes posttranslational modifications with their important impact on the function of various transporters. Chapter 11 covers the various classes of blockers for the dopamine transporter with detailed discussion of structural determinants, and Chapter 12 describes the use of various in vivo imaging techniques and ligands for biogenic amine transporters, in particular the dopamine transporter in the human brain. In Chapter 13, the final chapter, the focus is on dopamine transporter changes in human brain as a result of cocaine administration, with both in vitro and in vivo imaging approaches.

A number of differences between this second edition and the original book can be highlighted. First, the more basic cloning and pharmacological information on monoamine transporters, previously in Chapter 1, can now be found in Chapters 3 and 4. The previous topic of regulation of serotonin transporters (currently Chapter 1, previ-

ously Chapter 2) has been widened to include all three biogenic amine transporters, and it now emphasizes phosphorylation and trafficking phenomena. Three new chapters on biogenic amine transporters have been added: Chapter 3 on chimera and site-directed mutagenesis studies, Chapter 4 on gene organization and the relationship of polymorphisms with psychiatric and other complex human diseases, and Chapter 5 on transgenic animals carrying altered genes for plasma membrane monoamine transporters. Current excitement about channel properties of transporters is covered in Chapters 2 and 8 regarding electrophysiological studies on cloned monoamine and amino acid transporters, respectively. The new chapter on transporter imaging, Chapter 12, entirely focuses on human results including effects of aging and brain injury, and changes in schizophrenia, phobia, drug abuse, and other complex human diseases. Chapter 13 covers changes in dopamine transporters in human brain as a result of cocaine exposure as previously, with added discussion of in vivo imaging approaches for the dopamine transporter included in this update. The previous final chapter detailing the role of biogenic amine transporters in in vivo and in vitro monoamine release studies has been omitted as more recent material on in vivo results is now presented in the new Chapter 5.

The authors of the present chapters have been instrumental in advancing our knowledge of transporters by their experimental and conceptual contributions to the field, and I feel fortunate to have been able to join all their forces together in this second edition. I thank Paul Dolgert, Tom Lanigan, Sr., Elyse O'Grady, Craig Adams, and Mark Breagh at Humana Press for allowing the opportunity of a second edition, and I hope the book will continue to be used as a popular resource in the field.

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Mechanisms of Biogenic Amine Neurotransmitter Transporters

Gary Rudnick

1. INTRODUCTION

The biogenic amine transporters, as described in Chapters 1, 3, and 5 of this book, terminate the action of released biogenic amine neurotransmitters. These transporters utilize norepinephrine (NE), dopamine (DA), and serotonin (5-HT), and are referred to as NET, DAT, and SERT, respectively. Interruption of their function by agents such as antidepressants and stimulants causes profound changes in mood and behavior. In addition to their importance in regulating the extracellular concentration of neurotransmitters, these proteins are fascinating molecular machines that utilize the energy from transmembrane ion gradients to accumulate intracellular neurotransmitters. The pharmacology and molecular biology of these proteins is well covered in Chapters 1, 3, 5, and 11. This chapter focuses on the mechanism of neurotransmitter transport. We are still a long way from completely understanding how these proteins work. However, recent advances have given us further insight into this process, and encourage the hope that current and future research will provide a more complete understanding of the transport mechanism.

2. NA, K, AND CL IONS: COFACTORS FOR TRANSPORT

Like many mammalian plasma-membrane transport systems, the biogenic amine transporters require the presence of external Na^+ ions. This phenomenon was observed first for epithelial transporters, such as those for glucose and amino acids (1). The transport of NE into peripheral nerve endings was also found to be Na^+ -dependent (2), as was 5-HT transport into platelets (3). When synaptosomes were established as an experimental system for studying presynaptic mechanisms, the Na^+ dependence of neurotransmitter uptake

became firmly established, as each system in turn demonstrated its dependence on extracellular Na^+ (4–9). In all these cases, replacement of Na^+ with any other ion results in a loss of transport activity.

The importance of this Na^+ requirement became more apparent as the energetics of transport were studied. The Na^+ requirement provided a way to understand how the energy of ATP hydrolysis drives transport. ATP is utilized by the Na^+ pump to move Na^+ ions out of—and K^+ into—the cell. By incorporating Na^+ into the transport reaction, the neurotransmitter transporters couple the energy released by ATP hydrolysis to the downhill Na^+ flux that accompanies transmitter accumulation (Fig. 1). Because K^+ is pumped by the ATPase, it is not surprising that some neurotransmitter transporters also utilize internal K^+ in the transport process. The most notable are the 5-HT and glutamate transporters (10,11), although other systems may also take advantage of K^+ concentrated inside cells by the Na^+ pump. The requirement for internal K^+ can be fulfilled by H^+ in the case of the 5-HT transporter (12), but efforts to demonstrate this phenomenon with glutamate transporters have resulted in the opposite conclusion—that H^+ equivalents were moving with the flow of substrate rather than against it (13).

Another consequence of Na^+ pump action is a result of the fact that three Na^+ ions are pumped out for each two K^+ ions pumped into the cell, leading to the generation of a transmembrane electrical potential. In itself, this potential can be used by neurotransmitter transporters, but more specifically, the potential leads to the loss of Cl^- ions from the cell. Many neurotransmitter transporters utilize this asymmetric Cl^- distribution as a driving force. With the exception of glutamate transporters, all of the neurotransmitter transporters and many related transporters require Cl^- as well as Na^+ (14). For this reason, the neurotransmitter transporter gene family is often referred to as the NaCl-coupled transporter family (15). The glutamate transporters represent a separate gene family.

2.1. Transport and Binding Requirements

One advantage to the study of biogenic amine transporters, relative to other members of the family, is that high-affinity ligands are available. Tricyclic antidepressants such as imipramine and desipramine bind to SERT and NET, and have been used to investigate the ion dependence of the binding process. SERT requires both Na^+ and Cl^- ions for maximal [^3H]imipramine binding (16), although repeated attempts to demonstrate any effect of K^+ have been negative. Similar results have been obtained with [^3H]desipramine and NET (17). Antidepressant binding differs from trans-

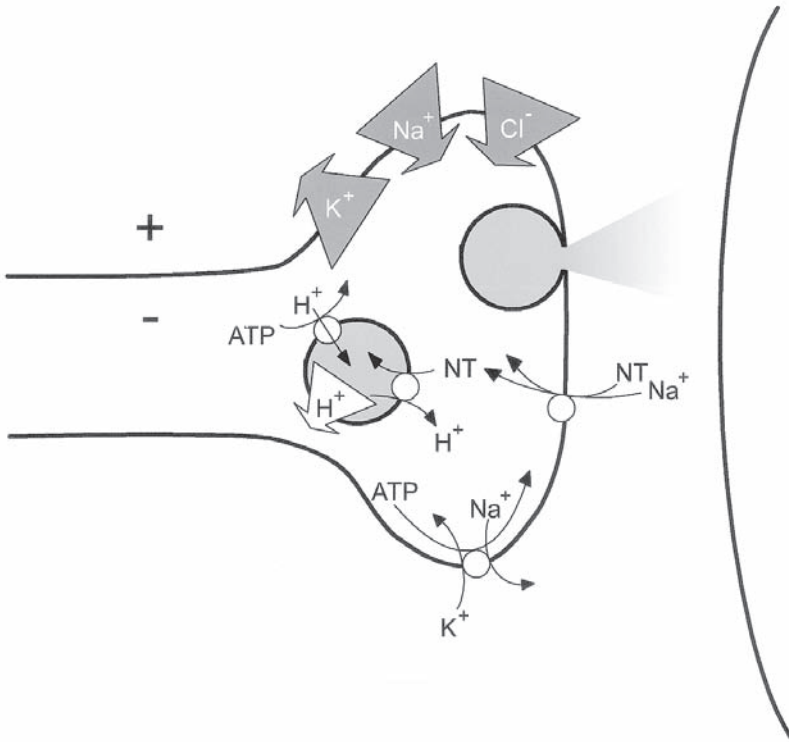


Fig. 1. Neurotransmitter recycling at the nerve terminal. Neurotransmitter (NT) is released from the nerve terminal by fusion of synaptic vesicles with the plasma membrane. After release, the transmitter is transported across the plasma membrane by a Na^+ -dependent transporter in the plasma membrane. Transmitter delivered into the cytoplasm is further sequestered in synaptic vesicles by a vesicular transporter using the transmembrane H^+ gradient as a driving force. This driving force, shown by the arrow pointing in the direction of downhill H^+ movement, is generated by an ATP-dependent H^+ pump in the vesicle membrane. The Na^+ and K^+ gradients across the plasma membrane are generated by the Na^+/K^+ -ATPase. This enzyme also creates a transmembrane electrical potential (negative inside) that causes Cl^- to redistribute. Neurotransmitter transport across the plasma membrane is coupled to the Na^+ , Cl^- , and K^+ gradients and the membrane potential generated by the ATPase.

port, however. The Na^+ dependence of [^3H]imipramine binding to SERT and imipramine inhibition of transport were both sigmoidal, suggesting that two or more Na^+ ions participate in the reaction (18). In contrast, the Na^+ dependence of 5-HT transport and 5-HT inhibition of [^3H]imipramine bind-

ing showed simple saturation behavior consistent with the involvement of only one Na^+ ion in 5-HT binding and transport (18). Similar results were obtained with NET. Transport is a simple saturable function of Na^+ (19), but [^3H]desipramine binding shows a sigmoidal Na^+ dependence (17).

When binding of other ligands was examined, the difference between substrates and inhibitors became even more obvious. Paroxetine and the cocaine analogs CFT and β -CIT bind to SERT and inhibit transport. This binding process was stimulated by Na^+ but not by Cl^- (20–22), although Cl^- is required for transport. The DA transporter also binds cocaine and its analogs (23,24), and has demonstrated a different ion dependence for transport and β -CIT binding (24). For both SERT and DAT, β -CIT binding was inhibited when Na^+ was replaced with Li^+ but did not require Cl^- , and for both transporters, β -CIT binding was inhibited at low pH (24). In contrast, the binding of 5-HT or DA, was stimulated by Cl^- and not affected by low pH (24), although neutralization of DA at high pH decreases its binding to DAT (25). The presence of Li^+ ions apparently favors a conformation of SERT with a lower affinity for cocaine and its analogs, but with similar 5-HT affinity (25a). These studies and others clearly indicate that inhibitor binding may be similar, in some aspects, to substrate binding. However, the two processes are distinct in their ionic dependence. Despite the differences, interactions between inhibitors and substrates at 5-HT, NE, and DA transporters are competitive, at least in equilibrium-binding studies. Thus, a single binding site, or a set of overlapping binding sites, could account for substrate and inhibitor binding.

The sensitivity of β -CIT binding to displacement by substrate has allowed measurements of substrate binding under conditions which preclude direct substrate-binding measurements. Using [^{125}I] β -CIT, 5-HT binding to SERT was measured in the absence of Na^+ and Cl^- , and the individual effects of these ions was determined (22). Although Cl^- stimulated 5-HT binding by itself, Na^+ alone actually decreased 5-HT-binding affinity. Maximal 5-HT affinity was observed only in the presence of both Na^+ and Cl^- , suggesting that 5-HT binds to the transporter together with these two ions (22).

3. ION GRADIENTS DRIVE BIOGENIC AMINES ACROSS THE MEMBRANE

3.1. Influence of Ion Gradients on 5-HT Transport

Studies with synaptosomes and platelets have indicated that the biogenic amine transport systems possess an impressive ability to concentrate DA, NE, and 5-HT. However, these preparations contained intracellular amine

storage organelles (synaptic vesicles or dense granules) that sequester most of the intracellular amine. The ability of the plasma-membrane transporters to concentrate their substrates was not appreciated until platelet membrane vesicles were shown to accumulate internal 5-HT to concentrations hundreds of fold higher than the external medium when appropriate transmembrane ion gradients were imposed (26). These vesicle experiments demonstrated conclusively that the plasma-membrane transporters generated gradients of their substrate amines, using the energy of transmembrane Na^+ , Cl^- , and K^+ ion gradients.

3.1.1. Na^+

When a Na^+ concentration gradient (out > in) was imposed across the vesicle membrane in the absence of other driving forces, this gradient was sufficient to drive 5-HT accumulation (26). Coupling between Na^+ and 5-HT transport follows from the fact that Na^+ can drive transport only if its own gradient is dissipated. Thus, Na^+ influx must accompany 5-HT influx. Na^+ -coupled 5-HT transport into membrane vesicles is insensitive to inhibitors of other Na^+ transport processes such as ouabain and furosemide, supporting the hypothesis that Na^+ and 5-HT fluxes are coupled directly by the transporter (26,27). Many of these results have been reproduced in membrane-vesicle systems from cultured rat basophilic leukemia cells (28), mouse brain synaptosomes (29), and human placenta (30).

3.1.2. Cl^-

The argument that Cl^- is cotransported with 5-HT is somewhat less direct, as it has been difficult to demonstrate 5-HT accumulation with only the Cl^- gradient as a driving force. However, the transmembrane Cl^- gradient influences 5-HT accumulation when a Na^+ gradient provides the driving force. Thus, raising internal Cl^- decreases the Cl^- gradient, and inhibits 5-HT uptake. External Cl^- is required for 5-HT uptake, and Cl^- can be replaced by Br^- ; to a lesser extent, by SCN^- , NO_3^- , and NO_2^- ; and not at all by SO_4^{2-} , PO_4^{3-} , and isethionate (27). In contrast, 5-HT efflux requires internal but not external Cl^- (27). The possibility that Cl^- stimulated transport by electrically compensating for electrogenic (charge moving) 5-HT transport was ruled out by the observation that a valinomycin-mediated K^+ diffusion potential (interior negative) was unable to eliminate the external Cl^- requirement for 5-HT influx (27).

3.1.3. K^+

The ability of internal K^+ to stimulate 5-HT transport was not immediately obvious, for two reasons. First, there was no absolute requirement for

K^+ in transport, and, second, no Na^+ cotransport system had ever been shown to be coupled also to K^+ . Initially, we proposed that a membrane potential generated by K^+ diffusion was responsible for driving electrogenic 5-HT transport (26). However, subsequent studies showed that K^+ stimulated transport even when the membrane potential was close to zero (11,31). In the absence of a K^+ gradient, the addition of 30 mM K^+ simultaneously to both the internal and external medium increased the transport rate 2.5-fold (31). Moreover, hyperpolarization of the membrane by valinomycin in the presence of a K^+ gradient had little or no effect on transport. Two conclusions were drawn from these results. First, the transport process was likely to be electrically silent. Second, since the K^+ gradient did not seem to act indirectly through the membrane potential, it was likely to act directly through exchange with 5-HT.

A study of the pH dependence of 5-HT transport revealed the reason that 5-HT transport still occurred in the absence of K^+ . A study of the pH dependence of 5-HT transport. In the absence of K^+ , internal H^+ ions apparently fulfill the requirement for a countertransported cation (12). Even when no other driving forces were present ($NaCl$ in=out, no K^+ present), a transmembrane pH difference (ΔpH , interior acid) could serve as the sole driving force for transport. ΔpH -driven 5-HT accumulation required Na^+ and was blocked by imipramine or by high K^+ (in=out), indicating that it was mediated by the 5-HT transporter, and not the result of non-ionic diffusion (12). From all of these data, it was concluded that inwardly directed Na^+ and Cl^- gradients, and outwardly directed K^+ or H^+ gradients served as driving forces for 5-HT transport (Fig. 2).

3.1.4. Electrical Consequences

Although studies using platelet plasma-membrane vesicles provided direct evidence that 5-HT transport was electrically silent (11,31), evidence relating the membrane potential to 5-HT transport has been mixed in other systems. Bendahan and Kanner (28) found that 5-HT transport into plasma-membrane vesicles from rat basophilic leukemia cells was stimulated by a K^+ diffusion potential. However, other workers studying plasma-membrane vesicles from mouse brain and human placenta concluded that 5-HT transport in these tissues was not driven by a transmembrane electrical potential ($\Delta\psi$, interior negative) (32,33).

One might expect that electrogenicity could be easily tested if cells expressing the 5-HT transporter could be directly impaled with microelectrodes. This has been done by Mager et al. (34), using *Xenopus* oocytes injected with 5-HT transporter mRNA, with somewhat surprising results. A simple prediction is that current should not flow across the membrane during

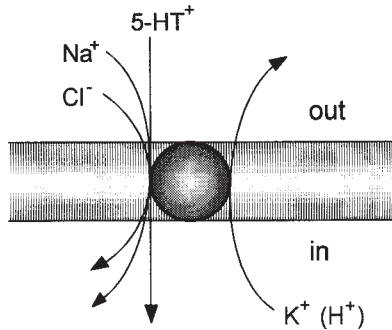


Fig. 2. Driving forces for 5-HT transport. Na^+ and Cl^- on the outside of the cell are transported inward with the cationic form of 5-HT. In the same catalytic cycle, K^+ is transported out from the cytoplasm. In the absence of internal K^+ , H^+ ions take the place of K^+ . The energy released by downhill movement of Na^+ , Cl^- , and K^+ provides the driving force for 5-HT accumulation against its concentration gradient.

5-HT transport if the transporter is electroneutral. In fact, a 5-HT-dependent current has been measured, but closer inspection of its properties suggests that it does not represent electrogenic 5-HT transport, but rather, a conductance that is stimulated by transport. The key finding is that the transport-associated current is voltage-dependent. Thus, the inward current increases as the inside of the cell becomes more negative. In the same cells, however, $[^3\text{H}]$ 5-HT transport is independent of membrane potential. Therefore, it is very unlikely that the voltage-dependent current represents the 5-HT transport process. Instead, as discussed in **Subheading 7**, the current results from a newly discovered ion-channel property of neurotransmitter transporters.

3.2. 5-HT Dopamine and Norepinephrine Transporters use the Protonated Form of the Substrate

Because 5-HT, DA, and NE exist primarily in the protonated form at physiological pH, many researchers have assumed that these substrates are transported as cations. However, a small fraction of these amines exist in the neutral or zwitterionic form at neutral pH, and it is important to assess the possibility that these forms are the true substrates for transport. In the case of the vesicular monoamine transporter (VMAT), the ionic form of the substrate is a matter of some controversy. Different investigators have reached opposite conclusions (35–37). If the neutral form were transported by the plasma membrane biogenic amine transporters, one consequence might be that the K_m for transport would be pH-dependent. As the pH increases, the mole fraction of biogenic amine in the neutral or zwitterionic form will increase sharply, but below pH 8.0–9.0, the majority of the sub-

strate will be in the cationic form and the mole fraction of that form will not change significantly. The K_m for total substrate (cationic and neutral) will therefore appear to decrease if the neutral form is the substrate but will be pH-independent if the cationic form is transported. Results with the 5-HT and NE transporters (38,39) show no change in K_m with pH, and suggest that the cationic form is the true substrate. An extensive study of dopamine transporter concluded that the cationic (and possibly also the zwitterionic) form of DA was the likely substrate (25).

There is another consequence if the neutral or zwitterionic form is transported. The substrate would need to dissociate a H^+ ion before transport, and then would bind H^+ after transport. By imposing a transmembrane pH difference (ΔpH), the equilibrium amine distribution across the membrane could be influenced. In seeming agreement with this prediction, 5-HT accumulation by platelet plasma-membrane vesicles is increased (in the absence of K^+) when the vesicle interior is acidified (12). However, this phenomenon represents the ability of H^+ to replace K^+ in 5-HT countertransport, and not an influence on 5-HT protonation. In the presence of K^+ , ΔpH does not stimulate 5-HT uptake, although this should occur if the neutral form of 5-HT is the true substrate (12).

3.3. Overall 5-HT Stoichiometry

The number of Na^+ , Cl^- , and K^+ ions transported with 5-HT transporter has been estimated by imposing known Na^+ , Cl^- , and K^+ concentration gradients across the plasma membrane as a driving force, and measuring the 5-HT concentration gradient accumulated in response to that driving force at equilibrium. This is essentially a thermodynamic measurement, balancing a known driving force against a measured gradient of substrate. Technically, such measurements require that the imposed ion gradients are relatively stable, so that the available driving force is known at a given time after imposition of the ion gradient.

3.4. Transport Kinetics can Suggest, but not Determine, Stoichiometry

Kinetic techniques have also been used to assess the Na^+ , Cl^- , and K^+ stoichiometry for 5-HT, NE, and DA transport. One technically simple technique is to measure the dependence of transport rate (or its kinetic determinants K_m or V_{max}) on Na^+ , Cl^- , or K^+ concentration, and to calculate a Hill coefficient for that ion. Using this analysis for the 5-HT and NE transporters yields an n of one for both Na^+ and Cl^- in membrane vesicles (40–42), where initial rates of transport showed a simple hyperbolic dependence on Na^+ or Cl^- —consistent with a $Na^+ : Cl^- : \text{substrate}$ stoichiometry of 1:1:1.

However, steady-state kinetics do not necessarily provide accurate information on cotransport stoichiometry (43). It is possible that more than one Na^+ ion is required for substrate binding or even translocation (as reflected in the Hill coefficient calculated from rate measurements), but that only one of those Na^+ ions is actually cotransported. It is also possible that a substrate is cotransported with two Na^+ ions, but that the affinities or rates of association of the two Na^+ ions are so disparate that the initial rate of transport is dependent on only the weaker binding or slower associating of the two, leading to an apparent Hill coefficient of 1. These difficulties are inherent in any kinetic method, whether transport is measured directly by tracer flux, or indirectly by measurements of electrical currents that may accompany transport. Thus, the dependence of transport rate on the concentration of a given ion may suggest a transport stoichiometry, but cannot provide proof for it.

A still more direct method is to measure the flux of driving ions as well as the flux of substrate. Usually the basal levels of ion fluxes are too fast relative to the rates of substrate transport, but in a purified, reconstituted system, Kanner and colleagues were able to measure Na^+ and Cl^- flux along with GABA flux by the GABA transporter. In this case, in which both thermodynamic and direct kinetic data exist, both methods indicate a $\text{Na}^+:\text{Cl}^-:\text{GABA}$ stoichiometry of 2:1:1 (63, 39, 40).

3.5. Thermodynamic Approach

Because kinetic approaches may be experimentally difficult or misleading, it is essential to confirm the stoichiometry by a thermodynamic measurement. In the thermodynamic method, known Na^+ , Cl^- , or K^+ concentration gradients are imposed across the plasma membrane as a driving force, and the substrate concentration gradient in equilibrium with that driving force is measured. By varying the concentration gradient of the driving ion, and measuring the effect on substrate accumulation, the stoichiometry can be calculated. For a simple system in which two solutes, A and B, are cotransported, a plot of $\ln(A_{\text{in}}/A_{\text{out}})$ vs $\ln(B_{\text{out}}/B_{\text{in}})$ gives the B:A stoichiometry as its slope. As a special case, if the stoichiometry is 1:1, then a plot of $A_{\text{in}}/A_{\text{out}}$ vs $B_{\text{in}}/B_{\text{out}}$ will be a straight line. Using this method, a 1:1 coupling was determined for 5-HT transport with both Na^+ (18) and K^+ (26). The Cl^- stoichiometry was deduced from the fact that 5-HT transport was not affected by imposition of a $\Delta\psi$ (interior negative), and was therefore likely to be electroneutral. Because 5-HT is transported in its cationic form (12,39), only a 5-HT $^+:\text{Na}^+:\text{Cl}^-:\text{K}^+$ stoichiometry of 1:1:1:1 is consistent with all the known facts. Obviously, this analysis requires an experimental system such as membrane vesicles, where the composition of both internal and external media can be controlled. This method also relies on the ability to

measure, or at least to estimate, an equilibrium substrate gradient under conditions in which the ion gradients are known (43).

3.6. Each Transporter has a Characteristic Coupling of Ion Flux to Substrate Flux

3.6.1. Norepinephrine Transport

Although no membrane-vesicle systems containing the DA transporter have been described, two plasma-membrane vesicle systems have emerged for studying NE transport: the placental-brush-border membrane (42) and cultured PC-12 cells (47). Harder and Bonisch (47) concluded that NE transport into PC12 vesicles was coupled to Na^+ and Cl^- and was electrogenic, but they failed to arrive at a definitive coupling stoichiometry because of uncertainties about the role of K^+ . According to their analysis, stimulation of NE influx by internal K^+ resulted either from direct K^+ countertransport as occurs with SERT (31), or from a K^+ diffusion potential which drives electrogenic NE influx, as with GAT-1 (48). Ganapathy and colleagues (42) studied NET-mediated transport of both NE and DA into placental-membrane vesicles (DA is utilized by NET as a substrate) (49). They reached similar conclusions regarding ion coupling, but also were left with some ambiguity regarding K^+ . In fact, the effects of ions on NET-mediated DA accumulation were similar to those observed with SERT-mediated 5-HT transport in the same membranes, and the two activities were distinguished only by their inhibitor sensitivities (19). Part of the difficulty in interpreting and comparing these data stems from the fact that they were obtained in various cell types, with unknown and potentially very different conductances to K^+ .

Two further problems made it difficult to interpret previous data on NET ion coupling. Both previous studies assumed that the cationic form of the catecholamine substrate was transported (42,47), and did not consider the possibility that the neutral or zwitterionic form was the true substrate. Moreover, previous studies estimated NET stoichiometry using kinetic rather than thermodynamic measurements. The number of Na^+ ions cotransported with each catecholamine substrate was estimated from the dependence of transport rate on Na^+ concentration (42,47).

The author and colleagues established LLC-PK₁ cell lines stably expressing the biogenic amine transporters SERT, NET, and DAT, as well as the GABA transporter GAT-1. Using these cell lines, we characterized and compared the transporters under the same conditions and in the same cellular environment (49). One attractive advantage of LLC-PK₁ cells is that it has been possible to prepare plasma-membrane vesicles that are suitable for transport studies (50). We took advantage of this property to prepare mem-

brane vesicles containing transporters for GABA, 5-HT and NE, all in the same LLC-PK₁ background. These vesicles should have identical composition, except for the heterologously expressed transporter. Moreover, these vesicles are suitable for estimating equilibrium substrate accumulation in response to imposed ion gradients. This property allowed us to define the ion-coupling stoichiometry for NET, using the known stoichiometries for GAT-1 and SERT-mediated transport as internal controls.

The results for SERT and GAT-1 are consistent with previously reported determinations of ion-coupling stoichiometry. For NET, accumulation of [³H]dopamine (DA) was stimulated by imposition of Na⁺ and Cl⁻ gradients (out > in) and by a K⁺ gradient (in > out). To determine the role that each of these ions and gradients play in NET-mediated transport, we measured the influence of each ion on transport when that ion was absent, present at the same concentration internally and externally, or present asymmetrically across the membrane. The presence of Na⁺ or Cl⁻, even in the absence of a gradient, stimulated DA accumulation by NET, but K⁺ had little or no effect in the absence of a K⁺ gradient. Stimulation by a K⁺ gradient was markedly enhanced by increasing the K⁺ permeability with valinomycin, suggesting that net positive charge is transported together with DA. The cationic form of DA is likely to be the substrate for NET, since varying pH did not affect the K_m of DA for transport. We estimated the Na⁺:DA stoichiometry by measuring the effect of internal Na⁺ on peak accumulation of DA. Taken together, the results suggest that NET catalyzes cotransport of one cationic substrate molecule with one Na⁺ ion, and one Cl⁻ ion, and that K⁺ does not participate directly in the transport process (38).

3.6.2. Dopamine Transport

The DA transporter has a different ion dependence from that of SERT or NET. Although initial rates of DA transport were found to be dependent on a single Cl⁻, two Na⁺ ions were apparently involved in the transport process (49,51). Thus, the initial rate of DA transport into suspensions of rat striatum was a simple hyperbolic function of [Cl⁻], but depended on [Na⁺] in a sigmoidal fashion. These data are consistent with a Na⁺:Cl⁻:DA stoichiometry of 2:1:1. These differences in Na⁺ stoichiometry have been reproduced with the cloned transporter cDNAs stably expressed in LLC-PK₁ cell lines, indicating that they are intrinsic properties of the transporters and not artifacts caused by the different cell types used (49). Although the precautions discussed here prevent any firm conclusions regarding the ion-coupling stoichiometry of the DA transporter, the ion dependence differs from that of both SERT and NET, suggesting that each of these three biogenic amine transporters has a unique stoichiometry of coupling.

4. AMPHETAMINES ARE SUBSTRATES FOR BIOGENIC AMINE TRANSPORTERS

4.1. *Amphetamine Action*

Amphetamines represent a class of stimulants that increase extracellular levels of biogenic amines. Their mechanism differs from simple inhibitors such as cocaine, although it also involves biogenic amine transporters. Amphetamine derivatives are apparently substrates for biogenic amine transporters, and lead to transmitter release by a process of transporter-mediated efflux from intracellular stores (52–54). Both catecholamine and 5-HT transporters are affected by amphetamines. In particular, compounds such as p-chloroamphetamine and 3,4-methylenedioxymethamphetamine (MDMA, also known as “ecstasy”) preferentially release 5-HT (55,56), and also cause degeneration of serotonergic nerve endings (57). Other amphetamine derivatives, such as methamphetamine, preferentially release catecholamines.

4.2. *Actions at the Plasma-Membrane Transporter*

The process of exchange stimulated by amphetamines results from two properties of amphetamine and its derivatives. These compounds are substrates for biogenic amine transporters (53,58–60) and they are also highly permeant across lipid membranes (54,58,60). As substrates, they are taken up into cells expressing the transporters, and as permeant solutes, they rapidly diffuse out of the cell without requiring participation of the transporter. The result is that an amphetamine derivative will cycle between the cytoplasm and the cell exterior in a process that allows Na^+ and Cl^- to enter the cell and K^+ (in the case of SERT) to leave each time the protonated amphetamine enters. Additionally, a H^+ ion will remain inside the cell if the amphetamine leaves as the more permeant neutral form (Fig. 3). This dissipation of ion gradients and internal acidification may possibly be related to the toxicity of amphetamines in vivo. The one-way utilization of the transporter (only for influx) leads to an increase in the availability of inward-facing transporter-binding sites for efflux of cytoplasmic 5-HT, and, together with reduced ion gradients, results in net 5-HT efflux.

4.3. *Actions at the Vesicular Membrane*

In addition, the ability of amphetamine derivatives to act as weak base ionophores at the dense granule membrane leads to leakage of vesicular biogenic amines into the cytoplasm (54,58). Weakly basic amines are able to raise the internal pH of acidic organelles by dissociating into the neutral, permeant form, entering the organelle, and binding an internal H^+ ion. Weakly basic amines such as ammonia and methylamine have been used to

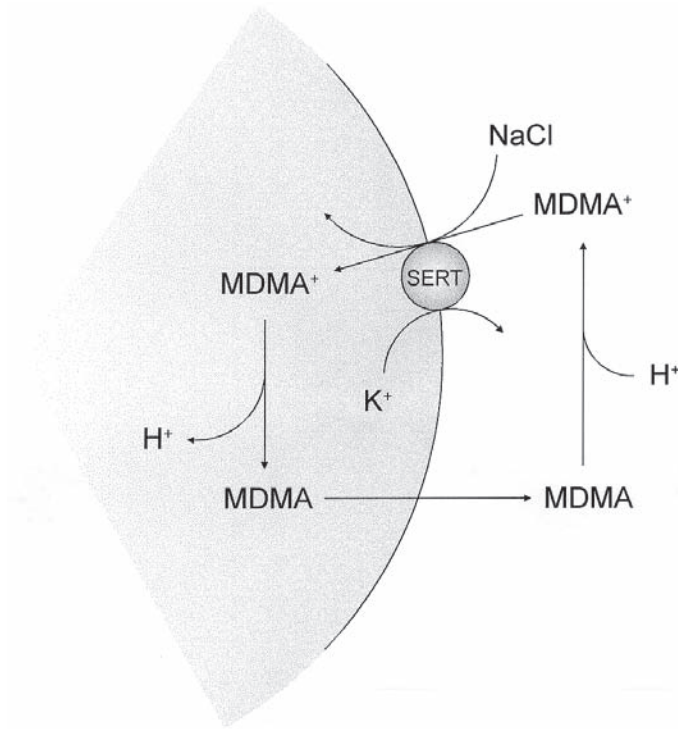


Fig. 3. Interaction of 3,4-methylenedioxyamphetamine (MDMA) with the 5-HT transporter. MDMA is a substrate for SERT, and, like 5-HT, is transported into cells together with Na^+ and Cl^- and in exchange for K^+ . Since it is membrane permeant in its neutral form, MDMA deprotonates intracellularly and leaves the cell, at which time it can reprotonate and serve again as a substrate for SERT. This futile transport cycle may lead to dissipation of cellular Na^+ , Cl^- , and K^+ gradients and acidification of the cell interior.

raise the internal pH of chromaffin granules, for example (61). Although amphetamine derivatives are certainly capable of dissipating transmembrane ΔpH by this mechanism, they are much more potent than simple amines when tested in model systems. For example, p-chloroamphetamine (PCA) and 3,4-methylenedioxyamphetamine (MDMA, ecstasy) are 5–10 times more potent than NH_4Cl in dissipating ΔpH in chromaffin-granule membrane vesicles (53,58). This result suggests that these compounds are crossing the membrane both in their neutral form, and also as protonated species. By cycling into the vesicle as an uncharged molecule and back out in the protonated form, an amphetamine derivative could act as a classical uncoupler to increase the membrane permeability to H^+ ions.

In addition to this uncoupling activity, amphetamine derivatives have affinity to the vesicular monoamine transporter, VMAT. Binding of various amphetamine derivatives to VMAT has been observed with both native and heterologously expressed VMAT (62). Despite the affinity of many amphetamines to VMAT, at least one compound, PCA, has no demonstrable binding to VMAT (60), despite its robust ability to release stored biogenic amines (58). Thus, the ability of amphetamines to dissipate vesicular pH differences is sufficient to explain their effects on vesicular release.

Sulzer et al. (63) have extended this hypothesis by measuring the effects of intracellularly injected amphetamine and DA. Using the *Planorbis corneus* giant dopamine neuron, they demonstrated that amphetamine could act intracellularly to release DA from the cell. Moreover, injections of DA directly into the cytoplasm led to DA efflux that was sensitive to nomifensine, suggesting that it was mediated by the plasma-membrane transporter. According to the hypothesis put forth by Sulzer et al. (63), amphetamine action at the vesicular membrane is sufficient to account for amphetamine-induced amine release. These results would also appear to explain the observation that the blockade of plasma-membrane transporters prevents the action of amphetamines (52,64).

4.4. The Amphetamine Permeability Paradox

If amphetamine derivatives act only by uncoupling at the level of biogenic amine storage vesicles, then classical uncouplers such as 2,4-dinitrophenol should act as psychostimulants like amphetamine. However, no such action has been reported for uncouplers or other weakly basic amines. Moreover, all of the amphetamine derivatives that we have tested bind to plasma-membrane amine transporters (53,58–60,65,66). What role could the plasma-membrane transporters play in amphetamine action? One possibility is that they serve merely to allow amphetamine derivatives into the cell.

An alternative possibility is that the ability of amphetamines to serve as substrates for plasma-membrane transporters is important in their action, even if the membrane does not constitute a barrier to amphetamine diffusion. In this view, futile cycling of the plasma-membrane transporter is induced by transporter-mediated influx followed by diffusion back out of the cell. As described previously, this process will lead to dissipation of Na^+ , Cl^- , and K^+ gradients, and could possibly acidify the cell interior. As a result of the lower gradients and the appearance of cytoplasmic binding sites following amphetamine dissociation on the cell interior, biogenic amine efflux via the transporter will be stimulated.

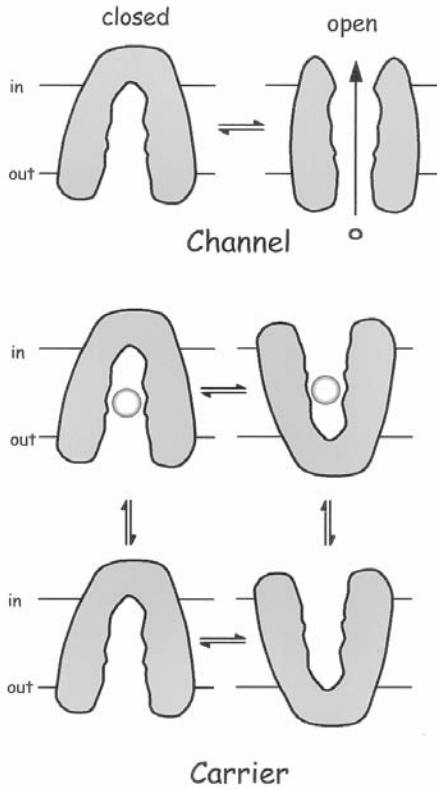


Fig. 4. Channels and carriers may have similar structures. A single structural model can account for transport by carriers and channels. In a channel, one or more “gates,” or permeability barriers, open to allow free passage for ions from one side of the membrane to the other. A carrier can be thought of as a channel with two gates. Normally, only one is open at a time. By closing one gate and opening another, the carrier allows a solute molecule, bound between the two gates, to cross the membrane.

The availability of knockout mice in which the gene encoding DAT was inactivated has helped to resolve this issue. Jones et al. (67) demonstrated that amphetamine caused dopamine efflux from synaptic vesicles, as judged by a decrease in DA released from the cell during exocytosis. However, DA was not released from the cell by amphetamine treatment (67). These results indicate that amphetamine does not require the plasma-membrane transporter for entry into the cell or action on synaptic vesicles. However, the plasma-membrane transporter was required for amphetamine to exchange with cytoplasmic DA.

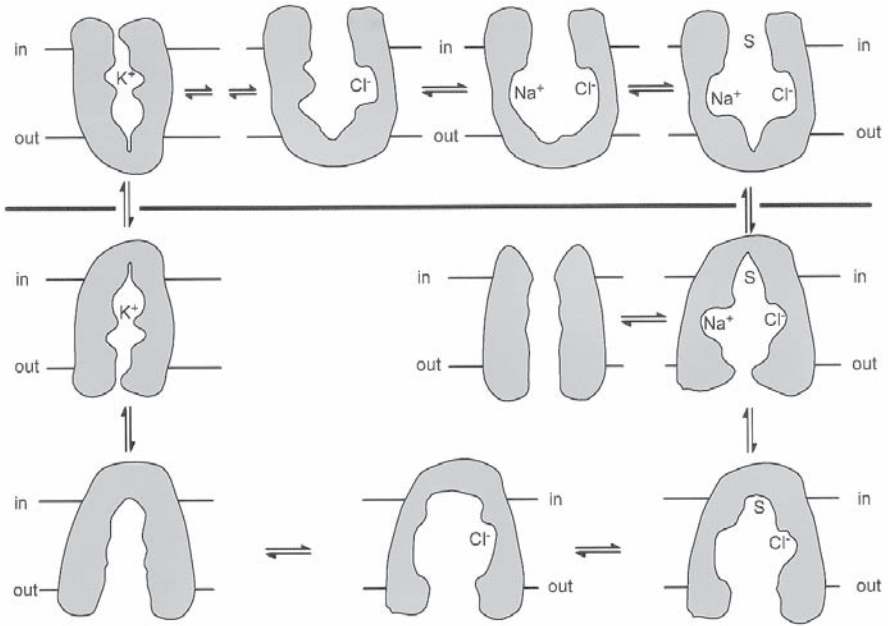


Fig. 5. Mechanism of 5-HT transport. Starting at the lower left and continuing counter-clockwise, the transporter binds Cl^- , 5-HT (S), and Na^+ . These binding events permit the carrier to undergo a conformational change to the form in the upper right-hand corner. This internal-facing form dissociates Na^+ , Cl^- , and 5-HT to the cytoplasm. Upon binding internal K^+ , another conformational change allows the carrier to dissociate K^+ on the cell exterior, generating the original form of the transporter which can initiate another round of transport by binding external Na^+ , Cl^- , and 5-HT. Also shown is a putative channel mode of the transporter, which could be responsible for uncoupled currents, in equilibrium with the loaded form of the carrier.

5. CONFORMATIONAL CHANGES AND TRANSLOCATION

5.1. Mechanisms for Ion Coupling

It is interesting to consider how the biogenic amine transporters, with predicted molecular weights of 60–80 Kd, are able to couple the fluxes of substrate, Na^+ , Cl^- , and K^+ in a stoichiometric manner. The problem faced by a coupled transporter is more complicated than that faced by an ion channel, because a channel can function merely by allowing its substrate ions to flow across the lipid bilayer. Such uncoupled flux will dissipate the ion gradients and will not utilize them to concentrate another substrate. However, the structural similarities between transporters and ion channels may provide a clue to the mechanism of transport (Fig. 4). Just as ion channels are believed to have a central aqueous cavity surrounded by amphipathic membrane-span-

ning helices, neurotransmitter transporters may have a central binding site which accommodates Na^+ , Cl^- , and substrate. The difference in mechanism between a transporter and a channel may be that a channel assumes open (conducting) and closed (nonconducting) states, yet a transporter can also assume two states which differ only in the accessibility of the central binding site. In each of these states, the site is exposed to only one face of the membrane, and the act of substrate translocation represents a conformational change to the state in which the binding site is exposed on the opposite face (Fig. 4). Thus, the transporter may behave like a channel with a gate at each face of the membrane, but only one gate is usually open at any point in time.

For this mechanism to lead to cotransport of ions with substrate molecules, the transporter must obey a set of rules governing the conformational transition between its two states (Fig. 5). For cotransport of Na^+ , Cl^- , and 5-HT, the rule would allow a conformational change when the binding site was occupied with Na^+ , Cl^- , and substrate. To account for K^+ countertransport with 5-HT, the reverse conformational change to external-facing form would occur only when the binding site contained K^+ . This simple model of a binding site exposed alternately to one side of the membrane or the other can explain most carrier-mediated transport. However, it makes specific predictions about the behavior of the transport system.

In particular, this model requires that the substrate is transported in the same step as Na^+ and Cl^- , but in a different step than a countertransported ion such as K^+ . As described previously, there is ample evidence from studies measuring binding of 5-HT and inhibitors that Na^+ and Cl^- bind to the transporter together with 5-HT (22). There is also evidence that 5-HT and K^+ are transported in different steps. The exchange of internal and external 5-HT does not require the K^+ -dependent step that is rate-limiting for net 5-HT flux. Thus, the steps required for 5-HT binding, translocation, and dissociation do not include the steps (where K^+ is translocated) that become rate-limiting in the absence of K^+ (31).

5.2. Structural Correlates of Conformational Changes

As detailed in Chapters 3, 4, and 6–8, the biogenic amine transporters were proposed to consist of alternating hydrophobic and hydrophilic stretches of amino-acid residues. The hydrophilic external-loop structures in SERT have been identified by site-directed chemical modification (68). The schematic mechanism of transport outlined in Fig. 5 ultimately must be reconciled with a structure that may contain 12 helical transmembrane segments connected by alternating extracellular and cytoplasmic loops. The conformational changes that are believed to convert substrate accessibility from extracellular to cytoplasmic and back again are triggered by binding

events that presumably occur within the transmembrane segments of a transport protein. However, the conformational changes themselves may involve more than transmembrane domains. Experiments with chimeric SERT constructs in which external-loop regions have been replaced with corresponding NET sequence emphasize the importance of these loops. Many of these constructs are severely defective in transport activity, although some chimeras that do not transport retain relatively normal ligand-binding activity (69,70). Cell-surface expression of these chimeras was at least as robust as wild-type. The loss of transport but not binding activity suggests that some of the external loops must be critical for steps other than binding, such as conformational changes that change the accessibility of the substrate-binding site. Despite the substitution of NET sequence for SERT, none of the chimeras with residual binding activity gained affinity for NET substrates or ligands. The similar binding selectivity between these mutants and wild-type suggests that the external loops do not play a significant role in forming the binding site for substrates and inhibitors.

In contrast, there is growing evidence that the transmembrane regions contain residues involved with substrate binding. Cysteine-scanning mutagenesis of the third transmembrane domain (TM3) of SERT revealed that at one position, Tyr-176, replacement with cysteine blocked transport activity (71). 5-HT and a cocaine analog were bound by Y176C, but the affinity was reduced. Modification of Cys-176 with the sulfhydryl reagent [2-(trimethylammonium)ethyl]methanethiosulfonate (MTSET) destroyed the remaining binding activity, and that inactivation was decreased in the presence of 5-HT or cocaine. Substitution of Ile-172 (one helical turn away from Tyr-176) with cysteine did not ablate transport activity, but I172C was also sensitive to inactivation of both transport and binding by MTSET, and like Y176C, 5-HT and cocaine protected against inactivation (71). These results suggest that Tyr-176 and Ile-172 may be close to, or within, a binding site shared by 5-HT and cocaine. Recent evidence indicates that after Cys-172 was inactivated by extracellular MTSET, it could be reactivated by intracellular thiols (72). Therefore, this position is accessible from both sides of the plasma membrane, although probably not simultaneously.

On the same predicted helical face of TM3 is another isoleucine, Ile-179, also one turn away from Tyr-176. Substitution at this position with cysteine led to sensitivity to MTSET. However I179C was not protected by 5-HT or cocaine (71). In both SERT and NET, in which the corresponding I155C mutant was occluded from reactivity after translocation of its catecholamine substrate (72), this position is conformationally sensitive. In many ways, SERT Ile-179 and NET Ile-155 behave as if they are part of the gate that closes to prevent substrate from dissociating after it is bound to the transporter.

6. BIOGENIC AMINE TRANSPORTERS ARE RELATED TO PUMPS, RECEPTORS, AND CHANNELS

6.1. *What is the Difference Between a Channel and a Carrier?*

The proteins responsible for accumulating biogenic amines are commonly called transporters, but they are more precisely referred to as carriers. Carriers and pumps are proteins that move solutes across membranes by a mechanism that requires a conformational change for every molecule or ion transported. Although carriers can couple the transmembrane movement of more than one solute, they are distinguished from pumps by their lack of coupling to metabolic energy. Pumps are quite similar to carriers in mechanism, but in addition to moving solutes, pumps also mediate a chemical reaction such as ATP hydrolysis, decarboxylation, or a redox or photochemical reaction that is coupled to the conformational changes in a way that utilizes the energy source to drive solute transport. Both carriers and pumps have the ability to use an energy source for solute accumulation, and this property sets them apart from channels, which only allow their substrate ion to flow down its electrochemical gradient.

The characteristic that most clearly distinguishes channels from carriers is the phenomenon of counterflow. In carrier-mediated transport it is common for the influx of a substrate into a cell or vesicle to be insensitive to, or even stimulated by, substrate efflux from the same cell. The movement of substrate in one direction does not interfere with movement in the opposite direction. This happens because the step that translocates substrate into the cell is distinct from the step that translocates substrate out. In a channel, where the two processes of influx and efflux both require the same aqueous pathway through the open channel, it is inevitable that influx will inhibit efflux and vice versa. An analogous situation in everyday macroscopic life is the difference between a stairway and an elevator in a multi-story building. If many people are rushing down the stairs, it is more difficult to climb upward against the crowd. However, if the same crowd is using the elevator to descend, it is even more likely to find an elevator waiting on the ground floor than when nobody is going down.

In this simplistic discussion, we have assumed that a given protein can be either a carrier or a channel, but recent evidence suggests that some proteins act as both. The 5-HT, NE, and DA transporters all seem to mediate uncoupled ion fluxes in addition to their ability to catalyze substrate accumulation (34,73–79). These transporters may not be unique in possessing more than one activity. There are reports that cystic fibrosis transmembrane regulator (CFTR) and multidrug resistance protein (MDR) can operate as both channels and pumps (80). It is important to distinguish the two activi-

ties. The ability of a biogenic amine carrier to conduct ions as a channel may represent a distinct activity of the protein. Although there might be conditions under which carrier and channel activity influence each other, uphill substrate accumulation cannot result from channel activity, and rapid ion conductance is unlikely to be caused by carrier activity.

6.2. How is a Carrier like a Receptor?

The triggering of conformational changes by substrate binding is one of the key events postulated to result in coupling of solute fluxes. For example, SERT does not transport 5-HT in the absence of Na^+ or Cl^- , presumably because all three solutes must be bound together on the transporter to trigger the conformational change that exposes them to the other side of the membrane. This process is not unlike the one in which a surface receptor undergoes a conformational change in response to agonist binding. In a receptor, that conformational change could open an ion channel or stimulate nucleotide exchange or enzymatic activity in an associated intracellular protein. In a carrier, the conformational change acts on the agonist (substrate) itself to change its accessibility from the internal and external faces of the membrane. From the perspective of evolution, carriers were much more important for primitive unicellular organisms (to ingest foodstuffs and excrete wastes) than channels and receptors. It is tempting to speculate that the structure and function of carriers was adapted to carry out the activities of channels and receptors as organisms developed the need for cell-cell communication.

7. TRANSPORTERS MEDIATE UNCOUPLED IONIC CURRENTS IN ADDITION TO TRANSPORT CURRENTS

7.1. What is the Relationship Between Uncoupled and Transport Current?

Recently, it has become apparent that in addition to the coupled flux of Na^+ , K^+ , and Cl^- , the 5-HT, DA, and NE transporters also catalyze uncoupled ion flux (34,73,79). Recent evidence for each of these three transporters demonstrates the appearance of ion-channel activity either by direct observation of single channels or by analysis of current noise (74,81,82). The uncoupled flux is therefore likely to represent events in which the transporter transiently operates like an ion channel instead of operating like a carrier. From the amount of current that flows with each channel event, and the frequency of channel events, it was possible to estimate that, for the 5-HT transporter, channels very rarely open relative to the number of times that a molecule of substrate is transported. Although there is evidence that substrates can move through the open channels (76,78), these channel events

are probably not an integral part of the transport process, but rather are analogous to a side reaction that occurs rarely in an enzymatic reaction. The uncoupled current, however, has important implications for the use of current recording techniques in transporter studies.

Studies that attempt to measure solute transport by recording the electrical current associated with transport can be confounded by the uncoupled ion flux. For example, although 5-HT transport is electroneutral, addition of 5-HT to oocytes expressing SERT leads to an inward current (34). Measurements of substrate accumulation, as opposed to current, depend on imposed ion gradients—and, in some cases, electrical potentials—but they are not likely to be influenced by uncoupled currents carried by the transporter. The total current will be the sum of any current associated with ion-coupled substrate transport, plus the uncoupled current that flows through any channels that open during the time of the measurement. Since the uncoupled current is frequently stimulated by substrate binding or transport (74,81,82), it is difficult to estimate the proportion of the total current that is coupled or uncoupled. The danger of assuming that all transporter-mediated current represents substrate flux is illustrated by the case of the glutamate transporter. This transporter has an associated uncoupled anion channel that is stimulated by glutamate (83,84). Previous studies of glutamate-stimulated current unknowingly assumed that the anion current actually represented glutamate influx, and prematurely concluded that anion efflux was coupled stoichiometrically to glutamate influx (85).

7.2. What do Electrogenic and Electroneutral Really Mean?

The recent demonstration that neurotransmitter transporters also mediate uncoupled ion flux (34,73,83,86), has cast a measure of confusion on the terms used to describe these proteins and their properties. If, as it seems likely, these transporters transiently form conductive channels through the membrane, a distinction must be made between the types of electrical currents caused by channel activity and substrate transport. The term “electrogenic” has traditionally been used to describe a coupled transport process in which net charge crosses the membrane. In the absence of ion gradients, an electrogenic transporter should generate an electrical potential in response to an imposed transmembrane substrate gradient. In contrast, the channel activity of such a transporter can only mediate energetically downhill ion flux. Thus, although SERT and NET both conduct ions by virtue of their intermittent channel activity, NET is an electrogenic transporter because it transports net charge with substrate (38), and SERT is electroneutral because the 5-HT transport cycle itself does not move net charge across the membrane (11).

7.3. What do Uncoupled Currents Tell Us?

Transporter-mediated currents are divided into three categories. The first and simplest are transport currents themselves, which result from electrogenic movement of ions during the transport reaction. Second, the individual steps in the transport cycle also may be associated with charge movement that appears as a transient current when the transporter redistributes between two states. These transient currents have been very useful in demonstrating and characterizing electrogenic binding of Na^+ to the GABA transporter (34,82,87–89). The third category are the uncoupled currents that seem, at least in some cases, to be the result of a channel activity of a transporter that normally functions only as a carrier. If, as recent results suggest, these channel openings occur very rarely with respect to the normal transport cycle, one might question whether they can tell us anything at all about the transporters. Perhaps they are just an epiphenomenon that has nothing to do with normal transport or physiological function. It is too early to tell for sure, but there are reasons to believe that these uncoupled fluxes may allow insight into the transporters that would be difficult to obtain in any other way.

The most interesting issue is the relationship between the aqueous pore through which ions permeate during channel activity and the pathway that substrates take as they are transported across the membrane. We think of the binding site for substrates as a potential channel through the core of the transporter, which is normally separated from one surface of the membrane or the other by permeability barriers (or gates). It is possible that if the barriers are both open, an aqueous channel through the membrane will be formed. If the ion channel does represent the substrate-transport pathway, it will provide support for the concept that transport substrates bind in a “channel” closed at one end or the other by gates. To learn if the transport pathway and the ion channel are identical, it would be helpful to know if substrates are bound during the time that the transporter is acting like a channel. Substrate stimulates channel activity by 5-HT, NE, and glutamate transporters (74,82,83). On the surface, this result might suggest that the channel opens when substrate is bound, and that the transport pathway, occupied by bound substrate, could not function as the channel. An alternative explanation, however, is that the transport of substrate and its release to the cytoplasm leave the transporter in a conformation with a higher probability of opening as a channel.

To distinguish between these possibilities, we must learn if the requirements for substrates to stimulate channel activity are at all different from those for transport. If conditions dictate that a substrate cannot be transported but does stimulate channel activity, it will suggest that substrate binding opens a separate channel, as in a ligand-gated ion channel. Alternatively,

if substrate binds within the ion channel, but dissociates from that site to leave the transporter in a state with a higher probability of opening for uncoupled ion flux, then substrate stimulation of channel activity would be observed only when substrate transport occurs.

Aside from the issue of the ion channel being the substrate site, there are other ways that channel activity may provide useful information about transporters. The properties of the channel—either its conductance, or opening or closing kinetics—may differ between states of the transporter. For example, the conductance of substrate-stimulated channels in a mutant 5-HT transporter is different from that of the channels observed in the absence of substrate (88). It may be possible to identify intermediates in the transport cycle with specific ion-channel properties. These properties could then be used to analyze the presence of those intermediates under specific conditions or in mutant transporters. The true impact of channel activity by transporters will be likely to modify our understanding of transport mechanism as more of its details are explored.

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