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PERMEATION PROPERTIES OF NEUROTRANSMITTER TRANSPORTERS

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PERSPECTIVES AND OVERVIEW

The Neurotransmitter Transporter Families

The neurotransmitter transporters belong to three known families of intrinsic membrane proteins (Figure 1). The energy for transport, which is often against the neurotransmitter concentration gradient, is derived from the cotransport (and in some cases the countertransport) of inorganic ions. Ion-transporting ATPases establish the concentration gradients for these ions.

Our initial classification is based on hydropathy analyses of primary amino acid sequences and on subcellular localization (Figure 1). However, we are also interested in relationships based on functional aspects, which we gather under the term permeation by analogy with similar aspects of ion channels (1, 2). For example, mammalian serotonin 5-hydroxytryptamine (5-HT) transporters, which we classify as Type 1, share little homology with the cloned glutamate transporters. Nonetheless, like the glutamate transporters (and unlike the other Type 1 transporters), the mammalian 5-HT transporter countertransports K^+. Therefore, in a later section of this review, we tentatively assign the mammalian 5-HT transporters to their own Type 1 subtype. Other possible classifications depend on the nature of the countertransported ions, specificity for organic substrates, and electrogenicity (3–6). Rapid progress in the cloning of these molecules ensures that any

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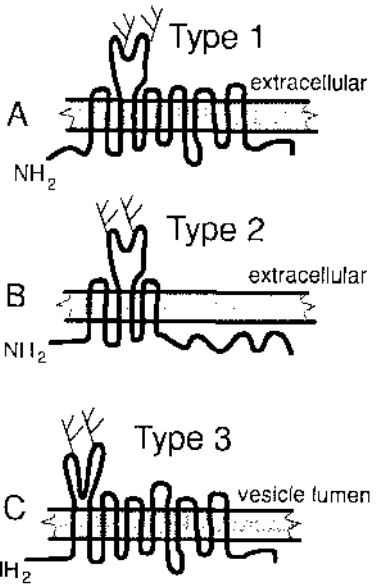

Figure 1 Cartoons of the putative transmembrane topology for three neurotransmitter transporter types. (*A*) Type 1, plasma membrane Na^+-coupled transporters. (*B*) Type 2, the plasma membrane glutamate transporter family. The number of transmembrane domains is uncertain (6–10). (*C*) Type 3, the vesicular H^+-coupled monoamine transporters.

survey of known members will be out of date before publication of this review; perhaps other families will be identified as well.

TYPE 1: THE PLASMA MEMBRANE NA^+/CL^- COUPLED FAMILY Members of one known family reside in the plasma membrane of neurons, glia and other cell types. The first cloned molecule in this family was the neuronal γ-aminobutyric acid (GABA) transporter, GAT1 (7), and cloning proceeded rapidly to more than a dozen known sequences, such as transporters for norepinephrine, serotonin, dopamine, glycine, and taurine (8). These transporters are characterized by 12 putative transmembrane helices and share 40–60% amino acid sequence homology; within this family, some subgroups have closer homology (4, 8). These proteins use Na^+ and Cl^- as cotransported ions (9a). Many other ion-coupled transporters have similar postulated transmembrane topology and functional and kinetic properties, but lack detailed sequence homology to neurotransmitter transporters (6)—a fact that poses interesting challenges for structure-function studies.

TYPE 2: THE GLUTAMATE FAMILY A second family of neurotransmitter transporters localized to the plasma membrane has three known highly homologous members, which transport excitatory amino acids and bear sequence homology to some bacterial transporters (10–12b) but not to the Type 1 transporters described above. The results of hydropathy analyses are ambiguous: the sequence data suggest a range of 6 to 10 putative transmembrane domains. Na^+ is cotransported as for Type 1 transporters; however, in contrast to the situation for Type 1 transporters, K^+ is countertransported. The identity of other co- and countertransported ions is not certain at present (13).

TYPE 3: THE VESICULAR FAMILY These transporters are localized to the membranes of synaptic vesicles, chromaffin granules, platelet dense granules, and other secretory vesicles. Four functionally defined H^+ neurotransmitter antiporters load transmitters into the vesicles in preparation for release. The organic substrates are either monoamines, glycine/GABA (14), acetylcholine (15), or glutamate (16); Cl^- may be involved in monoamine (17) and glutamate (16) transport. The monoamine transporter has been cloned and is presently represented by two isoforms: one is localized in the CNS and the other in the periphery (18–20). A putative acetylcholine transporter has been cloned as well (21).

These transporters have 12 putative membrane-spanning domains (Figure 1). They display no significant sequence homology with the Type 1 transporters, but they do resemble bacterial drug-resistance transporters (18, 19). The transporters are presumed to have a large extramembrane loop between the first two transmembrane segments, and this loop is oriented toward the vesicular lumen.

The synaptic vesicle proteins termed SV2A and SV2B (22–24) and p87 (25) have the same putative membrane topology as the vesicular transporters. However, their function and putative organic substrate have not been identified. Recent studies have examined the homologies among these proteins and suggested that the vesicular transporters are members of a superfamily termed major facilitator proteins (26, 27).

Analogies with Ion Channels

Figure 2 summarizes historical trends in models for transporter function over the past 30 years. Most present schemes are based on an alternating-access model. In this model the transporter has a central aqueous cavity, or lumen, containing binding sites for the ions and for the neurotransmitter. The transport involves a transition that exposes part or all of this lumen alternately to the extracellular and intracellular solution (5, 6, 28–32).

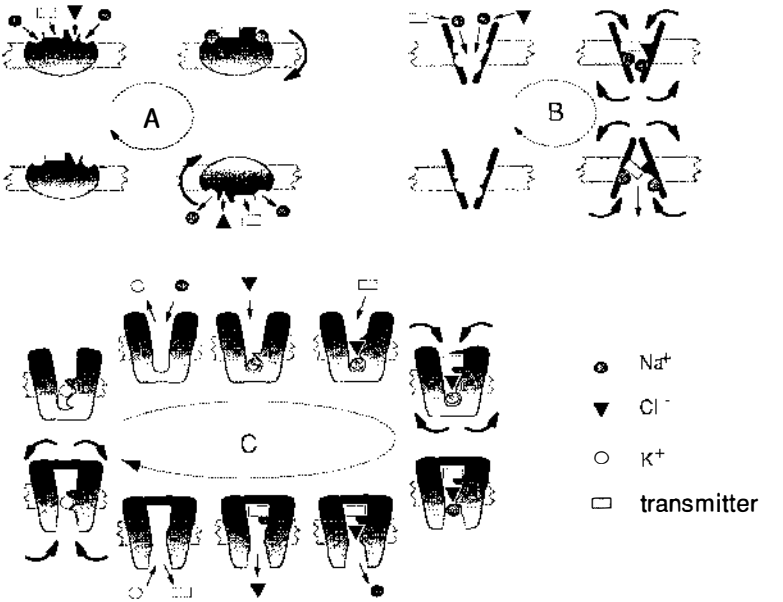

Figure 2 Cartoons summarizing historical trends in models for transporter function. Dashed arrows show the normal direction of the cycle, which brings the neurotransmitter from the extracellular space (at the top in all the cartoons) to the cytoplasm. Light arrows show binding and dissociation of ions; heavy arrows show conformational changes. Shaded circles are Na^+ ions; triangles are Cl^- ions; open circles are K^+ ions; and rectangles are neurotransmitter molecules. (*A*) A model based on rotation of the transporter molecule in the membrane, embodying the probable stoichiometry for GAT1. In light of modern knowledge that membrane proteins are quite large, have many membrane-spanning helices, and do not rotate in the indicated plane, researchers no longer consider this model valid. A carrier, resembling a low-molecular-weight antibiotic, is also considered an unlikely model. (*B*) A model that has the same stoichiometry as *A* but that incorporates the more modern concept of alternating access. When the appropriate binding requirements are met, the transporter undergoes a conformational change that alters the compartmentalization of the binding sites. (*C*) A more detailed alternating-access model that incorporates generally accepted stoichiometry for the 5-HT transporter and the ideas of Rudnick (32a). In this model, the substrates bind and dissociate in a specified first-in-first-out order, which is forced by a linear, channel-like lumen. This model allows for partial conformational changes associated with each binding step. Some experiments dispute the strictly ordered binding, however. The major conformational changes are channel-like gating events at either end of the lumen. Note the shading pattern, which emphasizes that the complete transporter protein does not undergo a rotation, in contrast to *A*.

This chapter employs a variant of the conceptual schemes discussed above based on our laboratory's history of ion channel research. Many previous authors have also compared transporters with channels (5, 6, 30–33). For channels, the substrates (permeative ions) are transported based on their electrochemical gradients and the fluxes of the various ions are partially

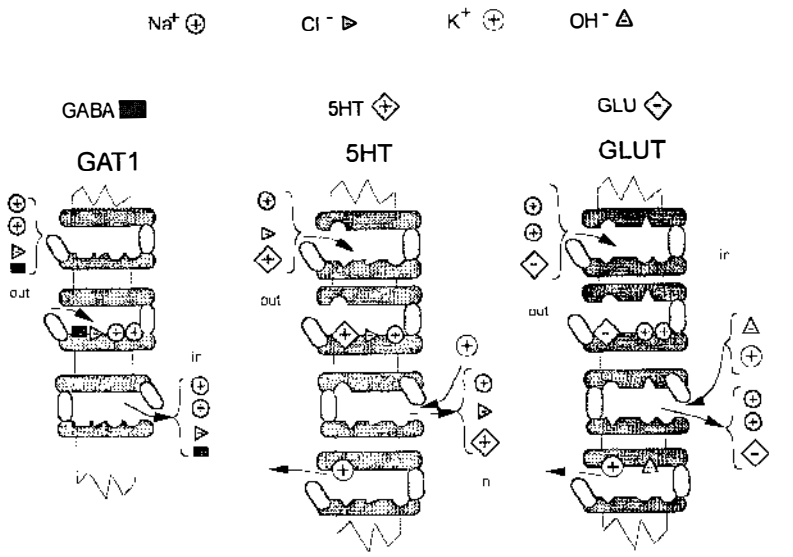

Figure 3 Cartoon of overall stoichiometry according to the alternating-access model for three well-studied transporters. The gates open and close when appropriate substrates are present at their binding sites. However, the stoichiometry of glutamate transport is not yet settled (see text).

independent. For ion-coupled transporters, the substrates include inorganic ions and organic molecules, such as transmitters; the flux of each substrate molecule is rather tightly coupled to the flux of the other substrates. Nevertheless, channels and transporters may share structural and functional components such as voltage- and ligand-dependent gating mechanisms, hydrophilic permeation pathways, and selectivity filters. We therefore view Na^+-coupled transporters as ion channels that have at least two gates (Figure 3). While the channel moiety has sites that bind, or perhaps merely accept, all the permeant substrates, the gates possess most of the (presently mysterious) properties that assure coupled transport. When all the substrates are in place, the gates undergo conformational changes, which account for the differences in compartmentalization of the substrates during the transport cycle. Such models evidently leave much unsaid in terms of real structure, but they do provide a conceptual framework for interpreting results.

Medical and Social Importance of Neurotransmitter Transport

Neurotransmitter transporters are the targets for many drugs of therapy and abuse. Cocaine is a psychomotor stimulant whose effects presumably result

from inhibition of transporters for the monoamines dopamine, serotonin, and norepinephrine (34, 35). Inhibition of the dopamine transporter appears to be most important for the reinforcing effects of cocaine in self-administration studies (34). The dopamine transporter is expressed only in dopaminergic neurons (36); therefore, researchers believe the effects of cocaine are most significant for the dopaminergic systems.

The monoamine transporters also serve as the therapeutic targets for tricyclic antidepressants. Individual drugs affect norepinephrine and serotonin transporters differently. New antidepressants, such as fluoxetine, are almost pure serotonin-uptake inhibitors. Specifically, serotonin-transport inhibitors have been used for treatment of depression, obsessive-compulsive disorder, and sleep and eating disorders (37). Patients diagnosed with major depression or obsessive-compulsive disorder often have fewer platelet or brain serotonin transport and binding sites. Similar reductions are seen after suicide attempts. These observations suggest a role for the transporter in neuropsychiatric disease (38, 39).

Amphetamine derivatives act on monoamine transporters in plasma membranes to release monoamines, presumably by heterologous exchange, and also dissipate the pH gradient in vesicles, causing monoamine release into the cytoplasm (40). The action on serotonin transporters is believed to underlie the effects of the widely abused drug 3,4-ethylenedioxy-methamphetamine (MDMA), or ecstasy (41).

Several authors have suggested that failure of glutamate uptake contributes to glutamate neurotoxicity (42). Altered glutamate transport has also been suggested as a potential cause of the neurodegenerative disorder, amyotrophic lateral sclerosis (ALS). For ALS, evidence indicates that a defect specific to glutamate transport is associated with the disease state. Glutamate transport is significantly reduced in synaptosomes prepared from the spinal cord, motor cortex, and somatosensory cortex of ALS patients (43).

We have discussed neurotransmitter transporters as manipulators of extracellular or intracellular transmitter levels. Transporters can also introduce transmitter analogues into the cytoplasm; these drugs can have therapeutic or toxic effects. For instance, the anticancer drug [^{131}I]meta-iodobenzylguanidine enters cells via the 5-HT transporter (44). Also, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a by-product of illicit synthesis of isomeperidine. MPTP first came to medical attention because it produces symptoms identical to Parkinson's disease, whose hallmark is destruction of dopaminergic neurons. Upon ingestion, MPTP is modified by monoamine oxidase to the toxic derivative MPP^+, which is then transported via the dopamine transporter into dopaminergic neurons. It is an inhibitor of NADH dehydrogenase and thus kills dopaminergic cells. This specific destruction of dopaminergic neurons has provided a model for the study of Parkinson's disease (45).

FUNCTIONAL ROLE OF NEUROTRANSMITTER TRANSPORTERS

Removing Transmitter

One role for neurotransmitter transporters is to terminate synaptic events by removing transmitter from the vicinity of pre- or postsynaptic receptors. In summary, extensive evidence shows that transmission at slow synapses (involving G protein–coupled receptors) is shaped by uptake. At fast synapses (involving ligand-gated channels), effects of uptake may also be crucial for correct transmission, but the effects are only revealed by more subtle experiments.

CATECHOLAMINES For dopamine and norepinephrine—two catecholamines whose receptors couple exclusively to one or more G proteins to produce slow synaptic effects (~1 s or slower)—blockade of uptake leads to abnormally large and prolonged synaptic events (46). Electrochemical detectors now give the best temporal resolution for measurements of extracellular catecholamine concentration and suggest that the half-life for norepinephrine is approximately 1 s in thalamus; the value for dopamine in the caudate nucleus is 33 ms. These half-lives are prolonged several-fold in animals treated with uptake inhibitors (47, 48).

GABA GABA uptake inhibitors both increase and prolong slow $GABA_B$ transmission (several hundred milliseconds) (49–51). Research suggests that GABA uptake directly influences the strength of excitatory synaptic transmission in the hippocampus by shaping the time course and amplitude of the diffuse $GABA_B$ inhibition (51).

Does uptake also control fast transmission to ligand-gated receptors? Studies on the importance of GABA uptake for $GABA_A$ transmission have exploited new potent, specific, and lipid-soluble uptake inhibitors such as Tiagabine and SKF-89976A, which bind directly to GABA transporters. Under conditions that produce overlapping release between neighboring sites, inhibition of GABA uptake does not change the amplitude of $GABA_A$ synaptic events but does prolong the decay phase, whose normal time constant is ~10 ms (49–51). Evidently, uptake mechanisms can prevent the GABA released within the synaptic cleft of a given synaptic site from diffusing onto $GABA_A$ receptors at neighboring synaptic sites.

SEROTONIN 5-HT participates in both fast transmission, by using ligand-gated channels, and slow transmission, by using G protein–gated receptors. Good evidence in both vertebrates (52) and invertebrates (53, 54) suggests that serotonin uptake inhibitors prolong slow transmission, but there are no studies on effects of such drugs for fast serotonergic transmission.

GLUTAMATE Inhibitors of glutamate uptake increase the amplitude of the relatively slow synaptic currents mediated by NMDA (N-methyl-D-aspartate) receptors (54a) but do not affect the amplitude or time course of faster (1–20 ms) synaptic currents mediated by non-NMDA receptors (54b). Eliasof & Werblin (55) have argued that extracellular glutamate in the amphibian retina is controlled over a longer time scale and a larger distance scale by the glial transporters than by the cone transporters, which would operate more quickly but only near a synaptic site.

ANALOGIES WITH ACETYLCHOLINESTERASE Data reviewed above suggest an analogy between effects of uptake inhibitors on slow and fast transmission and events at cholinergic synapses. The latter synapses rely upon the catabolic enzyme acetylcholinesterase (AChE) rather than on an uptake mechanism. AChE inhibition dramatically augments slow muscarinic transmission. AChE inhibition produces little change in the amplitude of fast nicotinic synaptic events but does prolong the time course of these events when many quanta are released simultaneously, which leads to overlap of neighboring events (56, 57).

Studies of nicotinic synapses show that AChE is present at relatively high densities [roughly $2500/(\mu m)^2$] in the synaptic cleft and that normal transmission requires such a density (57, 58). If neurotransmitter transporters play an analogous role at fast noncholinergic synapses, what are the implications of the fact that the turnover number of neurotransmitter transporters is many times lower than that of AChE (10^4/s)? Turnover number is an important consideration for slow synapses in which each transporter could deal with several transmitter molecules during a single synaptic event. However, we suggest that the important parameter for terminating a fast synaptic event is not the total turnover time for a transport cycle but simply the speed with which the population of transporters can sequester the population of free transmitter molecules in the synaptic cleft so that transmitter molecules cannot reach additional receptors. By this argument, one would expect to find that neurotransmitter-transporter densities for fast noncholinergic synapses (e.g. $GABA_A$, $5\text{-}HT_3$, and glycine) are comparable to that for AChE at nicotinic synapses. Although we lack quantitative data on this point, immunolocalization for GAT1 shows intense staining at the presynaptic terminals of cerebellar basket cell–Purkinje cell synapses (59).

Replenishing the Releasable Pool of Transmitter

A common tactic in neuroscience research is to assay transmitter release after addition of radiolabeled transmitter to the extracellular solution bathing neuronal cell cultures, or slices, or to the cerebrospinal fluid in intact animals. The label is taken up into cells and synaptic vesicles; it can then

be released by appropriate stimuli such as depolarization or Ca^{2+} influx. Nonetheless, the classical literature shows that only a minority of the released transmitter has followed the direct route of uptake followed by transport into vesicles. Most of the internalized monoamines are metabolized by pathways starting with monoamine oxidase or catechol-O-methyl transferase; and most of the intracellular GABA is converted to succinic semialdehyde by GABA transaminase (60, 61). Accumulation of the releasable transmitter derives from a combination of events: simple repackaging, resynthesis from breakdown products, and axonal transport of the new transmitter. The balance among these processes depends on acute stimulation, regulatory modifications of key biosynthetic enzymes, the developmental stage, and the particular tissue studied (60).

Choline is not itself a neurotransmitter, and the presumed sole function for the Na^+-coupled choline transporter is to replenish the intracellular pool of this neurotransmitter precursor; much of the choline transported by this molecule is acetylated to form acetylcholine, which is then transported into synaptic vesicles (60, 61). In the absence of cDNA cloning data, whether the choline transporter is a member of any known family is still unknown. Researchers now believe that a cDNA clone that conferred choline uptake in preliminary experiments encodes a creatine transporter (62).

Presumably, glial transporters primarily function in removal of transmitters, but whether glia release transmitters remains unclear. If they do release transmitters, transport-mediated release could be involved in replenishing the supply of transmitters in these cells. Another possibility is that glia maintain the extracellular concentration of glycine, which is necessary for NMDA transmission (71). Some glycine and glutamate transporters are expressed preferentially on glia in the CNS (11, 12, 63, 64).

Releasing Transmitter

Consider a neuron or glial cell that expresses a neurotransmitter transporter and is bathed in a constant concentration of the transmitter (perhaps because a nearby cell is steadily releasing transmitter). Consider also that an equilibrium distribution has been achieved in which the intracellular concentration is at the level determined by (*a*) the concentration gradients of the cotransported ions, (*b*) the membrane potential, and (*c*) the external concentration of the transmitter. If we assume that the internalized transmitter is not completely broken down or sequestered into vesicles, removal of external transmitters would lead to a new equilibrium concentration ratio that favors efflux. Studies have indeed detected such efflux from mammalian cells expressing GAT1 (65, 66).

Even in the continued presence of transmitter, a change in membrane potential in the concentrations of inorganic ions would lead to a new

equilibrium concentration ratio. A depolarization would favor efflux of the transmitter from the cytoplasm, particularly for transporters showing voltage-dependent permeation. GABA is released from horizontal cells in toad and catfish retina via this mechanism (67, 68); similar processes may function for GABA, dopamine, and norepinephrine release in the CNS (69, 70) and for the regulation of glycine concentration near NMDA receptors (71). Transporter-mediated release of glutamate from depolarized neurons or glia might lead to excitotoxic effects such as spreading depression (71). In general, transporter-mediated release would be distinguished from the well-documented vesicular release by the hallmarks of (*a*) Ca^{2+} independence and (*b*) nonquantal release.

CLASSES OF PERMEATION STUDIES

To evaluate the functions described above for neurotransmitter transporters, we need additional information that exploits and goes beyond the present accumulation of cDNA clones. Outstanding questions regarding function include (*a*) the detailed localization and density of the transporters; (*b*) the efficiency of transport (such as turnover number per transporter molecule) and the dependence upon cotransported ion concentration, membrane potential, and intracellular milieu; and (*c*) the possible modulation by intracellular messengers. Kanner & Schuldiner (72) have presented excellent analyses of the older literature on this topic. Many of the quantitative experiments used plasma membrane vesicles from rat brain or reconstituted vesicle systems, which Rudnick (73, 74) and Kanner (75–78) pioneered for the study of neurotransmitter transporters. Researchers have also employed primary brain cultures and, more recently, heterologous expression of cloned neurotransmitter transporters in various cell types to study transport. Analogous to studies on ion channels over the past decade, the combination of site-directed mutagenesis, heterologous expression, and high-resolution functional studies provides researchers with a productive strategy for obtaining information about transporters.

Equilibrium

Since the action of neurotransmitter transporters alters local substrate concentrations, one intellectually satisfying method of research is to study the transporter in a compartmentalized membrane system that allows control and/or measurement of all substrate concentrations in each compartment. The transporter is allowed to operate until each compartment contains an equilibrium concentration of neurotransmitter; the equilibrium concentration ratio (more precisely, activity ratio) is then studied as a function of the ionic concentrations. For a given transmembrane potential V_m, one has

$$V_m = \frac{RT}{F} \frac{\sum_x \ln \left(\frac{[X_o]}{[X_i]} \right)^{n_x}}{\sum_x n_x z_x},$$

where $[X_{o,i}]$ is the outside or inside concentration of substrate X, respectively; R, T, and F have their usual values; n_x is the number of molecules of X transported per cycle (positive for inward flow; negative for outward); and z_x is the valence of X. Equilibrium measurements have the advantage shared by all thermodynamic approaches: they give decisive information about the identity and stoichiometry of participating substrates.

Such an approach is approximated by experiments on membrane vesicles (79, 80). If, for example, the concentration gradient for the organic substrate depends on the nth power of the Na^+ activity gradient, then one concludes that each organic substrate molecule is transported in association with n Na^+ ions. For example, when this experiment was performed for GABA, the number found was $n = 1.5$, but various technical factors render this an underestimate of the actual number (79), and $n = 2$ is therefore the generally accepted number. Besides the difficulty of attaining appropriate experimental conditions, equilibrium measurements have the disadvantage of thermodynamic approaches: they give no information about intermediate steps or kinetics.

Flux Rates

If the flux of two radiolabeled substrates is measured simultaneously, conclusions can be drawn about relative stoichiometries. For instance, an early experiment on the Na^+/glutamate transporter in a neuronal cell line showed that two Na^+ ions were transported for each glutamate molecule (81); and later studies on GABA transporters reconstituted into lipid vesicles found that 2.58 Na^+ and 1.27 Cl^- ions were transported for each GABA molecule (9a).

An alternative approach is to study tracer flux rates as a function of the concentration of other substrates. This is not quite the same as the equilibrium method; for instance, three Na^+ ions might need to bind at some step, yielding a Hill coefficient of 3 for the dose-response relation between $[Na^+]$ and flux. If only one or two of these ions actually cross the membrane, however, equilibrium measurements would yield a concentration ratio for the organic substrate that varies with the first or second power of $[Na^+]$. Because most organic substrates are available in tritiated form at high specific activity, the flux rate experiment is usually the first to be done and yields much useful information.

Flux rates can also be performed under conditions leading to an exchange

rather than a complete cycle. The experimentalist can create a favorable environment for exchange of a given substrate A by maintaining high concentrations of one or more other substrates B and C on both sides of the membrane, thereby inhibiting dissociation of B and C. This procedure effectively limits the transporter to the right-hand states in Figures 2*B* and 2*C*, and these experiments give information about the binding order of substrates (72).

Ligand Binding

Ligand binding is often applied to the monoamine transporters, which have specific, high-affinity inhibitors. The binding is typically done with transporter-rich membranes rather than with soluble transport proteins. This approach is useful for quantitating sites. It also provides information about permeation mechanisms because some of the uptake inhibitors are thought to bind at or near the neurotransmitter site, thus stabilizing the transporter in an intermediate step (See Figures 2 and 3). Typically the binding increases with $[Na^+]$ and sometimes with $[Cl^-]$ as well, as though the neurotransmitter and one or more Na^+ and Cl^- ions are bound simultaneously, as the schemes of Figure 2 indicate (5).

Covalent labeling by specific ligands followed by solubilization and denaturing gel analysis has been applied to several Type 1 and 3 neurotransmitter transporters. Results generally show that the transporters have subunit molecular weights of 60–80 kDa and are glycosylated (4, 82–84). cDNA cloning and heterologous expression of the transporters later confirmed these conclusions. Covalent labeling has generated useful data about conformational changes and residues important for rabbit Na/glucose transporter function (6) and may become an important tool for studying neurotransmitter transporters as well. Ligand binding in conjunction with radiation inactivation experiments suggest that the rabbit Na/glucose transporter is a tetramer (85). Similar data for the neurotransmitter transporters are not yet available but will have important implications for the mechanism of permeation.

Electrophysiology

Another approach exploits the fact that most (but not all) transporters complete their cycle with a net transfer of charge; under typical physiological conditions, one net elementary charge enters the cell per transport cycle. Despite the fact that this charge transfer per transporter molecule ($1–10^4$ s^{-1}, depending on the transporter) is much less than the elementary current for a channel (typically $10^7 s^{-1}$), macroscopic currents can be measured with modern voltage-clamp techniques for nearly all transporters. This chapter

emphasizes such measurements, which were previously reviewed in the context of excitatory amino acid transporters (63).

Steady-state measurements of transporter currents can yield the following types of data. (*a*) In the usual application of electrophysiological analysis, steady-state currents are measured as a function of the nature and concentration of all substrates and the resulting information resembles that from radiotracer flux. (*b*) In conjunction with tracer fluxes, current measurements can provide stoichiometric information. (*c*) Electrophysiological measurements in conjunction with ligand binding to quantitate transporter number can yield turnover numbers. (*d*) Because transporter current would vanish at equilibrium, appropriately designed electrophysiological experiments could reveal how the equilibrium depends on substrate concentrations, membrane potential, and other factors. This thermodynamic measurement requires careful isolation of the transporter currents and is not often performed.

In most discussions of ion-coupled transport, a cycle with net transfer of charge is termed electrogenic and is associated with a voltage-dependent current during transport and a voltage-dependent rate of transport of organic substrate; examples include the Na^+-coupled transporters for glucose, GABA, glutamate, and Ca^{2+}. The relationship is not strict: we might imagine an electrogenic transporter in which voltage dependence vanishes under certain conditions because a voltage-independent step has become rate limiting. For instance, at low external [GABA], the GAT1 transporter has decreased voltage sensitivity, presumably because the rate-limiting step is a voltage-independent interaction between GABA and the transporter (86). Conversely, electroneutral uptake could be voltage dependent. This situation would occur if an intermediate step of the cycle, for instance the interaction between an ion and the transporter, is voltage dependent and becomes rate limiting.

The electrophysiological approach is useful for transient measurements as well. Consider that the net current for each individual transport cycle is the algebraic sum of a few individual steps involving charge movement, each of comparable magnitude to the entire cycle. These individual steps, shown schematically in Figure 2*B* or 2*C*, are the binding and dissociation of ions within the membrane field and/or conformational changes of the transport protein. Once the measurements have been optimized to reveal the steady-state currents that accompany transport, they can sometimes also reveal the transient currents resulting from the partial reaction steps. Indeed, a measurement of transient current for an individual step is theoretically possible even if the charge movement for the entire cycle sums to zero—that is, even if transport is electroneutral. Experimental strategies to reveal these transient currents are discussed below. In general, these experiments succeed

if (*a*) one can synchronize all the transporter molecules, so that a partial reaction step involving a charge movement results in a macroscopic transient current, and (*b*) the charge movement is slow enough to occur within the bandwidth of the measuring circuits.

PERMEATION PROPERTIES IN DETAIL

Type 1.1: GABA Transport

Early work on GABA transport used both flux rates and equilibrium measurements on plasma membranes from rat brain. This work consistently showed that two Na^+ ions and one Cl^- ion are transported for each GABA molecule under physiological conditions (Figure 3) (72). This section concentrates on recent electrophysiological studies of GAT1 function (summarized in Figure 4). The previous conclusions are supported by the findings that one elementary charge crosses the membrane for each GABA molecule transported (87) and that the Hill coefficient for the relationship between external [Na^+] and current is near two (86, 87).

For reasons described in a previous section, we sought experimental ways to measure macroscopic transient responses of the transporter currents after

Transport increases with:
extracellular [GABA] ($6 < K_{1/2} < 25$ μM)
extracellular [Cl^-] ($5 < K_{1/2} < 15$ mM)
extracellular [Na^+] ($20 < K_{1/2} < 500$ mM)
hyperpolarization

Stoichiometry: ~ 2 Na^+, 1 GABA, ? Cl^-

Rate-limiting steps:
[GABA] is rate-limiting at low [GABA]
Not voltage-dependent

At high [GABA], Na^+ can be rate-limiting
Voltage-dependent

At high [GABA], [Na^+], and [Cl^-],
Rate-limiting step is ~ 12 sec^{-1} (22 C),
not voltage-dependent.
Translocation? Ligand dissociation?

Charge movements:
Suppressed by SKF-89976A.
(Locks Na^+ onto transporter?)
Modified by GABA.
Reveal partial reactions.
Measure transporter density.
Tightly linked to Na^+ binding

Figure 4 Electrophysiological aspects of GAT1 function.

a rapid change in one or more conditions that affect transporter function. At least three perturbations are possible; each gives different information. (*a*) A perturbation could be performed by jumping the concentration of GABA (using rapid perfusion or caged GABA). This has not yet been accomplished. (*b*) An alternative time-resolved measurement would involve a step change in the concentration of another substrate, for instance Na^+ or Cl^-. Experiments using the Na^+/Ca^{2+} exchanger serve as an example (88). (*c*) We jumped the electrochemical gradient for Na^+ by jumping the membrane voltage, as shown in Figure 5. The current traces are taken from an actual experiment (86).

The cartoons of Figure 5 depict the molecular events that we believe produce these currents. Figure 5*A* represents transport when all the substrates are present. A jump in the membrane potential from −40 mV (top cartoons) to −100 mV (middle and bottom cartoons) produces an increased electro-

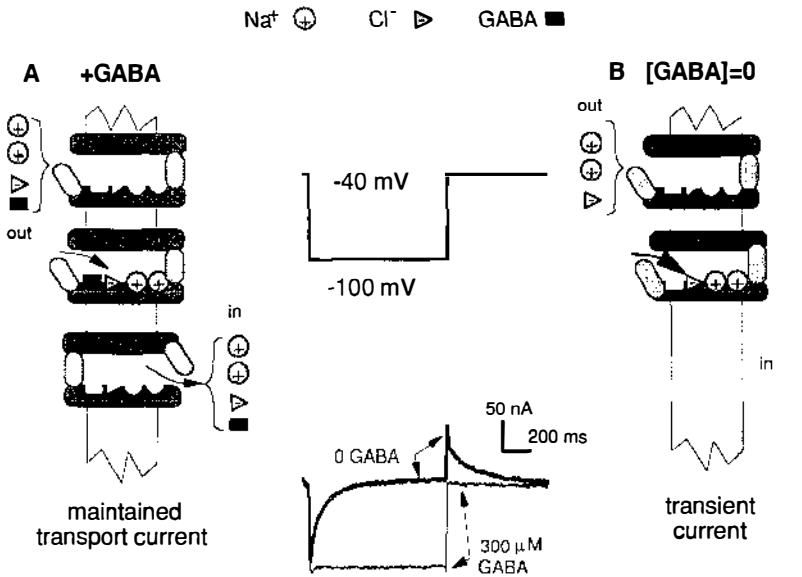

Figure 5 Cartoons of the relationship between steady-state currents and charge movements for the GAT1 GABA transporter. The experimental traces are from an experiment on *Xenopus laevis* oocytes expressing GAT1 (86). (*A*) All substrates (GABA, Na^+, and Cl^-) are present in the external solution. The voltage-clamp traces are shown as thin lines. (*B*) GABA is not present. Thick lines indicate the traces. The top cartoons in each case show the situation favored at depolarized potentials (−40 mV is shown as an example, although 0 mV would be more appropriate), and the middle and bottom cartoons show the situation favored at more negative potentials (−100 mV is shown as a example). The passive resistance and capacitance were eliminated by subtracting voltage-jump relaxations in a solution containing the GABA uptake inhibitor, SKF-89976A, and zero GABA (86).

chemical gradient for Na^+. This results in an increased inward current that accompanies an increased flux of GABA (lighter current trace). In the bottom cartoon, the substrates are released to the cytoplasm to complete the transmembrane current flow. The stoichiometry of $2Na^+/1\ Cl^-/1$ GABA ensures that each elementary charge crossing the membrane corresponds to the passage of one zwitterionic GABA molecule (87). Current continues to flow because all the substrates are continually present at the extracellular surface. When the voltage is returned to its previous value, the current simply returns to its original value as well.

The cartoon of Figure 5*B* depicts the absence of GABA and shows that only some steps can proceed. The actual voltage-clamp data are shown as thick traces. As in Figure 5*A*, Na^+ and perhaps Cl^- can bind at their sites. Because the Na^+ binding site is within the membrane field, this binding corresponds to a nct current into the membranc. Because the GABA molecule never arrives, the transporter cannot undergo the conformational change that releases all the substrates to the inside of the cell. The current stops flowing when all the Na^+ binding sites are occupied. A crucial aspect of this model is that when the voltage is jumped back to its original value, the Na^+ ions redistribute so that many return to the extracellular solution. Electrically, this redistribution is revealed as a transient current flow out of the cell. The transient currents at the beginning and at the end of the pulse were integrated to compute the total charge movement in the membrane, and the data showed that these charge movements are equal for the forward and reverse jumps between any two voltage levels. Thus, in the absence of GABA, the transporter currents are purely capacitive, because no Na^+ ions cross the membrane completely.

The data allow two independent and consistent estimates of the transporter's kinetics. 1. The transient currents decay with a time constant on the order of 100 ms, which suggests that a voltage-dependent step(s) takes about 100 ms in the absence of GABA. This step(s) appears to proceed at the same rate in the absence and presence of GABA, because the initial amplitude of the transient current in the absence of GABA roughly equals that of the maintained current in the presence of GABA. Therefore we can assume that the voltage-dependent step(s) becomes rate-limiting in the presence of saturating [GABA]. We believe that this rate-limiting step(s) could be the interaction between Na^+ and the transporter (including perhaps a tightly linked conformational change), but regardless of the nature of the rate-limiting step, it is a good assumption that an entire cycle would require on the order of 100 ms for completion at saturating [GABA]. 2. The total number of charges that move is evidently proportional to the number of transporters in the membrane. The constant of proportionality depends on how many charges move, and how far through the membrane (see below),

but the number is likely to be on the order of one and certainly less than three charges (2 Na plus 1 Cl) per transporter. Assuming the number is one, the saturating current may be divided by the number of transporters per oocyte to give a turnover number of $\sim 5\ s^{-1}$; extrapolating to infinite $[Na^+]$ gives $\sim 12\ s^{-1}$.

The actual locations of the various binding sites depicted in Figure 5 are not known. Analysis of the voltage dependence of the charge movements suggests that $\sum_i z_i \delta_i = 1.1$ where z_i is the charge on each ion ($z = +1$ for each of the two Na^+ ions and $z = -1$ for the Cl^- ion) and δ_i is the electrical distance from the external surface to which the ion penetrates. Also with regard to Figure 5, in normal Ringer solution, the charge movements are half-complete at -27 mV. The voltage for half-completion varies with external $[Na^+]$ and moves to more negative potentials as $[Na^+]$ decreases. The rate of this shift corresponds to a shift of ~ 42 mV per *e*-fold change in $[Na^+]$, as though δ_{Na}, the electrical distance at which Na^+ binds, is 0.55. No charge movements are detectable in zero external $[Na^+]$.

Although Cl^- is cotransported, the mechanism is not understood in detail. When external $[Cl^-]$ is varied, the charge vs voltage curve moves in the same direction as for $[Na^+]$ changes. This shift is opposite of that expected for simple binding of a Cl^- anion within the field. Therefore it seems more likely that Cl^- binds close to the membrane surface and that this binding increases the affinity of Na^+ for the transporter.

The steady-state transport currents are measured as a function of external [GABA], $[Na^+]$, and $[Cl^-]$. The dose-response relations are summarized by half-maximal concentrations as a function of voltage. As noted, the Hill coefficient for Na^+ exceeds one (1.5 in Ref. 86, 1.7 in Ref. 87); furthermore, the half-maximal concentration depends exponentially on membrane potential at a rate of *e*-fold per 42 mV. This result suggests that Na^+ binds within the membrane field. That equivalent voltage dependences are measured for the capacitive charge movements in the absence of GABA and for steady-state currents in the presence of GABA suggests that the same interaction between Na^+ and the transporter, at 55% of the electrical distance across the membrane, governs the two phenomena. Finally, the GABA uptake inhibitor SKF-89976A blocks the GABA-induced currents as well as the charge movements, probably by stabilizing a state in which Na^+ is bound to the transporter (86, 89).

OTHER MAMMALIAN GABA TRANSPORTERS Three other molecules that transport GABA have been cloned from the mammalian CNS and peripheral tissues (90–93). These transporters are 40–60% homologous in sequence to GAT1. The transporter cloned from kidney also transports betaine and

probably functions in osmoregulation (90). Uhl & Hartig (94) present a table comparing the names independently assigned to the same sequences by several groups. These transporters differ in their pharmacology of inhibition and K_m for the organic substrates (93). No detailed electrophysiological information is available yet for the newer members.

NONMAMMALIAN GABA TRANSPORTERS Physiological experiments reveal Na^+-coupled GABA transporters on crayfish stretch receptors (95–97) and on amphibian (98) and skate (99) retinal horizontal cells. Researchers have recorded transport-associated currents in each preparation but investigated and verified chloride dependence only for the skate preparation (99). In each of these nonmammalian preparations, the half-maximal GABA concentration is several times higher than for GAT1. Whether the invertebrate and amphibian retinal transporters correspond to one of the known cloned mammalian subtypes is unknown.

SIMILARITIES WITH THE RABBIT NA^+/GLUCOSE TRANSPORTER The permeation properties and proposed 12-helix topology of Type 1.1 neurotransmitter transporters are similar to those of many other plasma membrane Na^+-coupled transporters. Electrophysiological measurements of steady-state flux and capacitive charge movements reveal several similarities between the human Na^+/glucose transporter (100, 101) and GAT1. For instance, transport increases at more negative potentials and with increased $[Na^+]$. Transport also saturates under some conditions at high negative potentials. The half-maximal organic substrate concentrations are on the order of 10 μM and increase by several fold between -20 mV and -140 mV. Furthermore, charge movements have an apparent $z\delta \sim 1$ and shift to more negative potentials with decreased $[Na^+]$. Charge movements are also suppressed by addition of organic substrate or inhibitor. In addition, the transporters have a maximal turnover rate of about 5 s^{-1}. The Hill coefficient for $[Na^+]$ is greater than one, suggesting that two or more Na^+ ions are associated with each transport cycle. Finally, the interaction between Na^+ and the transporter is thought to be rate limiting under physiological conditions.

The permeation data for the rabbit Na^+/glucose transporter and those for GAT1 differ in three important ways. First, GAT1 but not the Na^+/glucose transporter depends on external Cl^-. Second, the Na^+/glucose transporter displays a small glucose-independent Na^+ flux leak, which appears as a decrease in current produced by a transport inhibitor; the experiments on GAT1 reveal no such currents. Last, the Na^+/glucose transporter but not GAT1 apparently has an additional voltage-dependent step involving a conformational change.

SIMILARITIES WITH OTHER NA^+-COUPLED TRANSPORTERS Researchers have observed the basic pattern of electrophysiological results, including sigmoid dependence of transport currents on [Na^+] and capacitive charge movements in the absence of organic substrate, for a *Drosophila Melanogaster* Na^+/serotonin transporter (102), a Na^+/myoinositol transporter (103, 104) and several other Na^+-coupled transporters (E Wright, personal communication; M Quick, JL Corey, J Guastella, N Davidson & HA Lester, unpublished observations). This experimental paradigm will evidently be a useful and general approach to transporter function.

Type 1.2: 5-HT Transport in Mammals

In the rat (105, 106, 107) and human (108, 109) genomes, a single gene encodes all known 5-HT transporters, including those in the brain and on platelets, placenta, and lung, although there is evidence for differential mRNA processing in human tissues (109). The presence of a single 5-HT transporter gene is a remarkable contrast to the extensive diversity among 5-HT receptors.

Researchers have studied the mammalian 5-HT transporter for several years for two major reasons. First, there is a rich repertoire of specific and high-affinity ligands for this transporter (110); these ligands interact with each other and with 5-HT in binding (see for instance, 111–113). And second, platelet membranes provide a plentiful and homogeneous source of transporter (73). The pharmacology and protein chemistry of this uptake system was quite advanced even before the transporter was cloned (5). Studies demonstrated that many uptake inhibitors bind with higher affinity in the presence of Na^+ as though the inhibitor binds at the neurotransmitter site and stabilizes the intermediate step that involves the simultaneous binding of Na^+ and the neurotransmitter (114). Likewise, the binding of 5-HT itself (115) and of some (114) but not all (116, 117) inhibitors is enhanced by Cl^-, which is consistent with cotransport of Cl^- (118).

We tentatively suggest that the permeation properties of mammalian 5-HT transporters justify their classification as a separate subtype, Type 1.2. The primary amino acid sequence is not a sufficient reason for classifying the mammalian 5-HT transporter separately from those we term Type 1.1, since its sequence is ~60% homologous to that of GAT1. However, the 5-HT transporter exhibits a different functional stoichiometry from GAT1 and other Type 1.1 transporters, as follows:

1. A K^+ ion is countertransported (119, 120); under some circumstances, a proton can substitute for K^+ (121).
2. Most studies show that the 5-HT transporter is electroneutral, that is, the number of charges entering and leaving are equal (reviewed in 5). However, at least one study (122) suggests that transport is electrogenic

because it depends on potential in plasma membrane vesicles from rat basophilic leukemia cells.

3. Most studies show that one Na^+ ion is involved. This conclusion is based on equilibrium measurements (80), the dependence of flux rate on [Na^+] (5), and the hyperbolic [Na^+] dependence of the binding of [^{3}H]paroxetine (117, 123) or the cocaine analogue β-CIT (115). However, at least one study reports that the Na^+ dependence of transport shows a Hill coefficient near two (124), and the Na^+ dependence of imipramine binding is sigmoid, as though more than one Na^+ ion is involved in the binding of these nontransported inhibitors (80). Perhaps Type 1.2 transporters do have two Na^+ binding sites, but one is not usually necessary for transport or ligand binding (G Rudnick, personal communication).

ELECTROPHYSIOLOGY OF MAMMALIAN 5-HT TRANSPORT Despite the fact that the complete 5-HT transport cycle was thought to involve no net transmembrane charge movement (see Figure 3), individual steps in transport should involve transient charge asymmetries. Therefore we undertook a series of electrophysiological experiments on the cloned rat 5-HT transporter expressed in *X. laevis* oocytes (125). The data are more complicated than one would expect for a simple electroneutral transporter: Evidence indicates at least two conducting states of the 5-HT transporter.

The first conducting state produces the transport-associated current, which is an inward current that occurs simultaneously (within 0.5 s) with 5-HT application to the oocyte. Like the uptake of [^{3}H]5-HT, this current depends hyperbolically on external [Na^+] and is suppressed by the 5-HT uptake inhibitor fluoxetine. However, the transport-associated current differs in one crucial aspect from [^{3}H]5-HT uptake: It is voltage dependent, varying by more than two-fold over the range from −30 to −80 mV, whereas the [^{3}H]5-HT uptake varies by less than 10% over this voltage range. A quantitative comparison of the transport-associated current to the number of 5-HT molecules transported is an important goal; among the complicating aspects of such an experiment are that [^{3}H] flux must be measured under voltage-clamp conditions and the efflux of possible neutral breakdown products such as 5-HIAA must be suppressed. Data to date indicate that the charge transfer and the [^{3}H]5-HT flux differ by at least a factor of 5.

A second conducting state, the transient state, was noted during voltage-jump experiments intended to reveal capacitive currents like those for Type 1.1 transporters. We found that jumps to potentials more negative than −100 mV produced large (several microamperes), transient (time constant, ~100 ms) inward currents. Uptake inhibitors suppress these transients; however, these currents differ in several ways from the capacitive currents measured with GAT1. (*a*) The 5-HT transient currents but not the GAT1

transients are inactivated by prepulses to negative potentials. (*b*) The 5-HT transients continue to grow at the largest negative potentials accessible (−180 mV), but the GAT1 transients level off at voltages more negative than −100 mV at normal [Na^+]. (*c*) The 5-HT transients do not reveal an equal and opposite outward charge flow when the potential is stepped back to the holding level. (*d*) The 5-HT transportcr transients arc supprcssed by 5-HT itself, but the GAT1 transients become maintained currents in the presence of GABA.

These observations are consistent with the view that the transient current represents a transient voltage-dependent opening of the gates in the absence of 5-HT. We doubt that this phenomenon operates under physiological conditions, but it has mechanistic significance in revealing a channel-like pathway through the transport protein.

We also measure a small steady-state inward leakage current (several nanoamperes) in the absence of 5-HT. It is possible that this current, which is suppressed by transport inhibitors, represents a third conducting state of the mammalian 5-HT transporter.

OTHER MAMMALIAN MONOAMINE TRANSPORTERS The mammalian transporters for dopamine and norepinephrine are slightly more homologous to the 5-HT transporter than to other Type 1 transporters (8). Because little is yet known about the permeation properties of the dopamine and norepinephrine transporters, we cannot yet classify them as Type 1.1, Type 1.2, or another subtype.

INVERTEBRATE SEROTONIN TRANSPORTERS At present, far more is known about 5-HT transport for mammals than for other organisms, but incomplete evidence suggests that the properties of invertebrate 5-HT transporters differ from those of mammalian 5-HT transporters. First, both the leech (54) and *D. melanogaster* (102) transporters show a sigmoid dependence on [Na^+] for the transport-associated current (M Quick, JL Corey, J Guastella, N Davidson & HA Lester, unpublished observations). This observation suggests that the two nonmammalian transporters may be classified as Type 1.1 transporters, unlike the Type 1.2 rat 5-HT transporter. Second, the *D. melanogaster* transporter depends on external Cl^- (102), whereas the leech transporter is independent of external Cl^- (54). Next, the half-maximal [5-HT] for the leech transporter is nearly 10-fold higher (54) than that for the mammalian transporter. In contrast to the mammalian 5-HT transporter, the uptake of [^{3}H]5-HT for the *D. melanogaster* transporter increases at more negative potential. This point has not been reported for the leech transporter; but in contrast to mammalian 5-HT transporters, the leech transporter shows no dependence on K^+. Finally, the *D. melanogaster* transporter displays voltage-dependent charge movements similar to those

for the Type 1.1 transporters. However, in all cases, transport-associated currents are measured and increase at more negative membrane potentials (54, 102, 125).

Type 2: Glutamate Transport

Studies have found no detectable amino acid homologies between the Type 2 glutamate transporter family and the Type 1 transporters, which is in accord with substantially different permeation properties. As of mid 1993, the most complete data for glutamate transporters come from native and reconstituted membrane vesicles from rat brain (72) and from whole-cell patch-clamp experiments on amphibian glial cells (Müller cells), as detailed below. However, less complete data for mammalian glia show similar phenomena (126, 127). Data for heterologous expression systems will soon become available and may extend the measurements to the time-resolved voltage-jump experiments that are proving fruitful for the Type 1 transporters.

Experiments suggest a stoichiometry of two Na^+ ions entering, one glutamate entering, one K^+ leaving, and one OH^- or HCO_3^- leaving (13, 72; see Figure 3). Under some conditions, a proton can substitute for at least one Na^+ ion (128). Neither external nor internal Cl^- is required. The requirement for internal K^+ has been observed both with membrane vesicles (11, 77, 78) and with studies on transport currents (129, 130; but see 33). Internal Rb^+ or Cs^+ can substitute for K^+. In accord with a scheme in which K^+ is countertransported during uptake under physiological conditions, external K^+ suppresses transport currents (10). There are no experiments combining measurements of current and radiolabeled glutamate flux.

Schwartz & Tachibana (33) found that relaxation kinetics of the glutamate transporter in salamander retinal glial cells were not resolvable following a light flash that released caged glutamate. Among the alternative explanations of this result, the one offered by the authors is that the turnover rate of the transporter exceeds $10^3\ s^{-1}$ at -66 mV. This is at least an order of magnitude higher than that inferred from purification and reconstitution of a rat brain glutamate transporter (33a). A turnover rate of $10^3\ s^{-1}$ is also two orders of magnitude higher than the rates observed for the Type 1 transporters and, if referred to the apparent maximal current densities of ~0.6 pA/pF at -66 mV (33, 129), corresponds to a transporter density of only $\sim 25/\mu m^2$. A small amino acid–independent inward Na^+ current with a Hill coefficient of ~3 for Na^+ is also present (33). The high turnover number and several other provocative results of that study led the authors to suggest that co-transport and uncoupled Na^+ flux are mediated by the same transporter and that "the transporter behaves, in some respects, more like a pore frozen in its open state than a carrier that undergoes a conformational change" (p. 77). This view is substantially different from the cartoons presented in

Figure 2 but does foreshadow our suggestion that there are conducting states for the Type 1.2 serotonin transporter.

The dose-response relation for the glutamate- or aspartate-activated current has the same shape at all membrane potentials between −20 mV and −100 mV. Equivalent statements are that the current-voltage relation has the same shape at all organic substrate concentrations and that the half-maximal concentration of ~20 μM for both glutamate and aspartate does not depend on potential (33, 131). These data are significant for the mechanism of permeation, for they imply that the transmitter does not bind within the membrane field.

In addition to L-glutamate, the compounds D- and L-aspartate, DL-threo-β-hydroaspartate, and several other substituted amino acids are also substrates for the transporter studied in Müller cells and for at least one of those cloned from brain (10). The most commonly used nonsubstrate inhibitors are kainate or dihydrokainate. Relative specificities for the organic substrates and for inhibitors vary by several fold in some cases among both native (132) and cloned (S Amara, personal communication) excitatory amino acid transporters. These differences may become important diagnostic tools for distinguishing among actions of the glutamate transporter subtypes.

Type 3: Vesicular Transport

Heterologous expression experiments will result in detailed characterization of the vesicular monoamine transporter. Initial data confirmed older studies showing that reserpine and tetrabenazine bind at distinct sites (133, 134). Johnson (135) and Edwards (17) have reviewed classical experiments that led to the conclusion that transport is electrogenic, so that accumulation is driven by the negative intravesicular potential as well as by the ΔpH. It is not clear whether the actual transported species is the neutral or protonated monoamine molecule (monoamine^0 and monoamine$^+$, respectively) so that the net overall stoichiometry would be either monoamine^0/1H$^+$ or monoamine$^+$/2H$^+$.

Electrogenic proton-coupled transport also characterizes the vesicular transporters for acetylcholine (15), for GABA/glycine (136), and perhaps for glutamate (16).

MODULATION OF PERMEATION PROPERTIES FOR TYPE 1 AND 2 TRANSPORTERS

Regulation and modulation of neurotransmitter transporters is potentially important in contexts as diverse as therapy, pathology, plasticity, and mechanism of permeation. We review the fourth point here. Consensus phosphorylation sites for protein kinases C and A reside in the amino and

carboxy termini of the members of the Type 1 and Type 2 neurotransmitter transporters (4, 8, 10–12). This observation suggests that activation of second-messenger systems could play a role in the regulation of these transporters in the nervous system, and this possibility is supported by evidence indicating that second messenger activation alters the activity of endogenous neurotransmitter transporters. For instance, in human platelets, 5-HT transport is decreased by acute treatment with phorbol esters (137).

GABA Transport

In glial cells, protein kinase C activation reduces GABA transport, but neuronal GABA uptake remains unchanged (138). GABA uptake in glial cells is also affected by activation of β-adrenergic receptors (139). It was therefore of interest to study modulation of cloned GABA transporters. Activation of protein kinase C increases the activity of GAT1 expressed in *X. laevis* oocytes, while inhibitors of protein kinase C and of Ca^{2+}/calmodulin-dependent protein kinase decrease GABA transport (JL Corey, MW Quick, N Davidson, HA Lester, unpublished results). The observed modulation results from changes in V_{max} but not K_m. This modulation persists for site-directed mutations lacking the three consensus phosphorylation sites for protein kinase C, thus suggesting an indirect effect of phosphorylation on transporter modulation.

Glutamate Transport

In primary cultures from rat cerebral cortex, phorbol esters produce a 50% increase in glutamate transport in glial cells while having no effect on glutamate transport in neurons (141). In contrast to the results with GAT1, this modulation may involve direct phosphorylation of the transporter. Activation of C-kinase by phorbol esters stimulates glutamate transport in HeLa cells heterologously expressing a cloned glial transporter, and this increase in activity can be abolished by mutation of a serine residue (142).

WHICH RESIDUES LINE THE PERMEATION PATHWAY?

The presumed cytoplasmic amino- and carboxy-terminal tails of Type 1.1 and 1.2 transporters are unlikely to be crucial for permeation, because (*a*) they are poorly conserved among transporters, (*b*) deleting these tails preserves function in some cases (107, 143, 144), and (*c*) chimeras bearing the N- and C-terminal cytoplasmic domains from the norepinephrine transporter and the bulk of the molecule from the 5-HT transporter function as

nearly normal 5-HT transporters (107). Chimeras involving these domains thus do not yet lead to an appreciation for the location of residues that are crucial in the permeation pathway.

Amara & Kuhar (4) and Giros & Caron (8) have summarized possible structural motifs that are common to many neurotransmitter transporters. Site-directed mutagenesis experiments have concentrated on (*a*) charged residues in membrane-spanning regions and (*b*) the residues that are conserved across many Type 1.1 and 1.2 transporters. Of the five GAT1 residues in group *a,* only Arg69 in helix 1 is required for function (145). An aspartate residue in helix 1 is required for binding of the cocaine analogue CFT and for transport (146), and an analogous residue in the 5-HT transporter is crucial for function (107). Given the limited information thus far obtained from this approach, investigators may now turn their attention to mutations of residues in class *b,* including uncharged residues that have been implicated in permeation properties of channels (2). Site-directed mutagenesis experiments point to a serine residue in helix 7 as important for the binding of dopamine itself, but not of cocaine analogues, to the dopamine transporter (146). These experiments were conceived and interpreted within the same tradition as earlier experiments showing that ligands bind to seven-helix receptors at points within the transmembrane portions of several helices. The fact that mutation of an identified serine residue abolishes C-kinase modulation may provide an important clue to the localization of the permeation pathway for Type 3 transporters (142).

We expect that the next few years will see rapid progress on the localization of the permeation pathway for neurotransmitter transporters. Many appropriate tools are in place: numerous homologous sequences, excellent pharmacological probes, and high-resolution electrophysiological measurements. Perhaps one of the mutations already available, or one under construction in one of the active neurotransmitter transporter laboratories, will provide the detailed data required for important new ideas.

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