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Topical Review

Neurotransmitter Transporters: Regulators of Function and Functional Regulation

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Introduction

Reliable chemical neurotransmission requires spatial and temporal control of neurotransmitter in the synaptic cleft. Neurotransmitter transporters, located on neurons and glia at or near the synapse, are key participants in this process. Historically, neurotransmitter transporters have been thought to participate by removing transmitter from the synaptic cleft; exciting new data reveal that this is only one route by which transporters regulate synaptic events. In addition, recent evidence suggests that transporters and transporter function can be regulated through multiple mechanisms. Taken together, these findings are the impetus for the present review, the title of which is chosen to denote the dynamic interplay among the factors that control neurotransmitter transporter function and the control of synaptic signaling by neurotransmitter transporters. Herein, we review (i) the ways in which neurotransmitter transporters are regulators of neuronal function and (ii) the ways by which the transporters themselves are regulated. We apologize at the outset for excluding a number of excellent scientific contributions from many different laboratories. We have deferred discussion of related topics including vesicular transporters, sodium-dependent glucose transporters, transporter classification, and transporter structure/function; other recent review articles cover these topics in detail.

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Transporters Regulate Synaptic Signaling

At first glance, the presence of transporters at or near the synapse, coupled with their role in transmitter removal, suggests that transporters are ideally situated to affect the magnitude and the time course of synaptic signaling. However, the empirical data are few, and carrier-related effects have been subtle. One difficulty with this simplified model of transporter-mediated effects on signaling comes from kinetic studies revealing that transport rates are slow: Based upon either voltage jump relaxation or transporter density measurements, unitary transport rates are on the order of 10/sec for the Na^+/Cl^--dependent transporter subfamily, including the GABA (Mager et al., 1993) and norepinephrine (NE) transporters (Galli et al., 1995), and 100–1000/sec for the Na^+/K^+-dependent glutamate transporter subfamily (Schwartz & Tachibana, 1990). Such slow turnover rates imply that transport might affect normal synaptic signaling only at ‘‘slow’’ synapses; i.e., synapses in which responses are mediated through G protein-coupled receptors and signaling occurs on a time scale of hundreds of milliseconds. However, uptake of transmitter may not be the only mechanism of transporter action. New evidence suggests that transporters can alter synaptic responses on a millisecond time scale by sequestering transmitter at its binding sites within the transporter (Diamond & Jahr, 1997), effectively serving as a diffusion sink. Such a mechanism would allow for transporter-mediated signaling effects at ‘‘fast,’’ ligand-gated ion-channel synapses as well.

GABA

In the guinea pig hippocampal slice preparation, both the amplitude and decay of the response mediated through

''slow,'' $GABA_B$ receptors are altered during pharmacological blockade of GABA transporters (Isaacson, Solis & Nicoll, 1993). In contrast, GABA transporter blockade alters only the decay phase of the ''fast,'' $GABA_A$ receptor-mediated response; there is little change in response amplitude (Isaacson, Solis & Nicoll, 1993). It is likely that these transporter effects are due to inhibition of GABA binding to the transporter, as opposed to transport, since many transport inhibitors prevent substrate binding.

Glutamate

The role of transporters in glutamate-mediated receptor signaling appear to be system-dependent. In hippocampal slice preparations, NMDA receptor signaling is prolonged and response amplitudes are increased by glutamate transport inhibition (Hestrin, Sah & Nicoll, 1990). At cerebellar climbing fiber synapses, and at synapses between parallel fibers and Purkinje cells, blockade of glutamate uptake also slows the decay of AMPA-mediated excitatory postsynaptic currents (EPSCs) (Barbour et al., 1994). However, similar blockade produces little effect on the decay phase of non-NMDA-mediated EPSCs at synapses between Schaeffer's collaterals and pyramidal cells (Sarantis et al., 1993). In certain preparations, transporter blockade also increases AMPA-mediated EPSC amplitudes (Hestrin, Sah & Nicoll, 1990) and AMPA-mediated miniature EPSC amplitudes (Tong & Jahr, 1994). Recently, Diamond and Jahr (1997) showed that the rise time of miniature EPSCs is affected by inhibition of glutamate transport, and that this result occurs within the first several hundred microseconds of the response. This effect is too fast to be mediated by transport and so the authors suggest that glutamate binding to the transporter acts as a sink for extracellular transmitter.

Amines

Little empirical evidence exists for physiological effects of transporters at adrenergic and dopaminergic synapses. However, two preparations demonstrate a role for the serotonin (5HT) transporter in synaptic transmission. In a rat dorsal raphe slice preparation (Pan & Williams, 1989), cocaine, an inhibitor of amine uptake, increases the time to peak and the time constant for decay of 5HT-induced postsynaptic potentials. In the leech, both the postsynaptic response amplitude and time course at the Retzius-P cell synapse are affected by 5HT uptake inhibitors (Bruns, Engert & Lux, 1993).

Transporters Can Regulate Neuronal Excitability

The transport of substrates through transporters is accompanied by the concomitant flux of ions, predominantly Na^+, K^+, and Cl^-. Indeed, for plasma membrane neurotransmitter transporters, Na^+ cotransport is required for substrate flux; cotransport of Cl^- and countertransport of K^+ are features of specific transporter subtypes. Classic models of neurotransmitter transport suggest a stoichiometric coupling of substrate and ions. However, the application of high-resolution electrophysiological techniques to the study of transport-associated currents generated by these ions has revealed these models to be incomplete, and has led to a revolution in our thinking about how neurotransmitter transporters operate and the functional roles of these membrane proteins. In addition to extending our understanding of transport rates, electrogenicity (net charge associated with the transport cycle), and the voltage dependence of transport (*see* Lester et al., 1994), electrophysiological measurements of transporter-associated currents have revealed two additional transport states: (i) nonstoichiometric ion fluxes during substrate transport beyond that which is predicted from the stoichiometries derived from isotope flux assays; and (ii) substrate-independent ''leak'' currents. These new data have led to an appreciation of the ''channel'' behavior of neurotransmitter transporters, and raise the possibility that transporters not only participate in substrate transport but may also regulate neuronal and glial excitability (*see* Sonders & Amara, 1996). Presently, several different models have been suggested to account for these new transporter properties (Sonders & Amara, 1996; Galli et al., 1997). Without attempting to adhere to any given model, we discuss these three transporter modes: the classic transporter, the ligand-gated ion channel, and the leak channel.

The Classic Transporter Mode

Both biochemical and electrophysiological data confirm that the transporter exhibits a ''classic transport'' mode that results in the translocation of substrate (neurotransmitter) across the plasma membrane. Hill coefficient estimates from substrate- and ion-concentration experiments suggest that this mode is stoichiometric with respect to cotransported ions. Given that these transporters also participate in other modes of ion conduction (*see below*), the evidence of a definable stoichiometric process places constraints on the mechanism of transporter permeation.

Based upon the ions that participate in substrate transport, one transport cycle can result in a net charge translocation. The GABA transporter GAT1 shows a net +1 inward charge movement because of the cotransport of two Na^+, one Cl^-, and one GABA molecule per cycle (Mager et al., 1993). NE transport stoichiometry is one NE, one Na^+, and one Cl^- (*see* Rudnick & Clark, 1993). Mammalian 5HT transport is electroneutral (*see* Rudnick

& Clark, 1993; Mager et al., 1994); however, invertebrate 5HT transporters are electrogenic (Bruns, Engert & Lux, 1993; Corey et al., 1994a; Galli et al., 1997). The various glutamate transporters show electrogenic uptake of +1 or +2 net charges based upon a stoichiometry involving two or three Na^+ ions, one H^+, one glutamate ion, and counter-transport of one K^+ ion (*see* Kanai, Smith & Hediger, 1993). The counter-transport of OH^- is also a part of the stoichiometric flux of some glutamate transporters (Bouvier et al., 1992).

The Ligand-gated Ion Channel Mode

Because of slow transporter turnover rates (*see above*), it is unlikely that the ion fluxes of the ''classic transporter'' mode contribute substantially to changes in membrane excitability. However, each of the transporter types described above also show nonstoichiometric ion fluxes in the presence of substrate. To illustrate this point, a simple experiment is to perform a radiolabeled transmitter uptake assay while simultaneously measuring transport-associated currents. Assuming only stoichiometric fluxes, the amount of charge that should cross the membrane, predicted from the amount of radiolabeled substrate that crosses the membrane, routinely underestimates (by several orders of magnitude) the amount of charge movement measured experimentally (Mager et al., 1994; Fairman et al., 1995; Galli et al., 1995; Galli et al., 1997). Qualitatively, such fluxes resemble those exhibited by ligand-gated ion channels.

The clearest examples of such uncoupled fluxes come from the subfamily of cloned glutamate transporters (EAAT1-EAAT4), because the nonstoichiometric current is carried by Cl^- ions; Cl^- is not required for the stoichiometric-related currents (Fairman et al., 1995). These nonstoichiometric currents are 50-fold larger than the stoichiometry-associated currents. Such nonstoichiometric currents are also present in heterologously expressed rat brain GABA transporter clones (Cammack, Rakhilin & Schwartz, 1994), in human dopamine (Sonders et al., 1997) and NE transporters (Galli et al., 1995; Galli, Blakely & DeFelice, 1996), and in 5HT transporters from both *Drosophila* (Corey et al., 1994*b*; Galli et al., 1997) and rat (Mager et al., 1994). Because of the difficulty in teasing apart the ''classic transporter'' mode from the ''ligand-gated ion channel'' mode, the particular ions that carry the charge during nonstoichiometric flux have yet to be determined for all transporters.

A central question is the extent to which the ''classic transporter'' and ''ion channel'' modes are mechanistically separate. For example, the Cl^- currents due to glutamate transporter function show characteristics of both ligand-gated ion channels (e.g., current direction reverses at the Cl^- equilibrium potential) and transporters (e.g., requires extracellular Na^+ in addition to glutamate) (Fairman et al., 1995). However, other data suggest more distinct processes. For the rat 5HT transporter, the stoichiometric currents are not voltage dependent, whereas the nonstoichiometric currents are voltage dependent. Estimates of stoichiometry from Hill coefficients for this transporter confirm the stoichiometry obtained biochemically, as if there is a ''classic transport'' mode; however, there is a concomitant flux of 5–12 charges per cycle (Mager et al., 1994). Application of standard channel analysis to transporters (Galli et al., 1996; Lin, Lester & Mager, 1996; Galli et al., 1997) suggests a ''ligand-gated ion channel'' of small conductance (less than 3 pS) and brief open times (less than 1 msec). Open probabilities increase with substrate (neurotransmitter) concentration, and, at hyperpolarized potentials (approximately −100 mV), single channel currents are approximately 0.5 pA.

The Leak Channel Mode

Contributing to their potential role in regulating membrane excitability, transporters also exhibit substrate-independent leak currents. For glutamate transporters (Kanai et al., 1995; Vandenberg et al., 1995) and the human NE transporter (Galli et al., 1995), this current is carried by Na^+. For other transporters, the current can be carried by Li^+ and K^+ (Mager et al., 1994; Cammack & Schwartz, 1996; Lin, Lester & Mager, 1996; Galli et al., 1997). Interestingly, Li^+ does not substitute for Na^+ in the other transporter modes, suggesting a third transporter permeation state. In addition, these leak currents are typically eliminated by application of substrate or by inhibitors of substrate transport. Patch-clamp studies reveal the conductance properties of the ''leak channel'' mode to be significantly larger than that seen for the ''ligand-gated ion channel'' mode. GABA transporter ''leak channels'' show low probability openings with currents of approximately 1 pA at −50 mV (Cammack & Schwartz, 1996). The ''leak channel'' conductance of the rat 5HT transporter is approximately 6 pS (Lin, Lester & Mager, 1996), at least twice the conductance of the ''ligand-gated ion channel.''

Several studies suggest that these three conducting modes of the transporter can contribute to membrane excitability in a physiologically relevant way. In the retina, the hyperpolarization of cone photoreceptors (Picaud et al., 1995) and bipolar cells (Grant & Dowling, 1995), necessary for transduction of visual information, is likely due to the Cl^- conductance associated with glutamate transporters acting in the ''ligand-gated ion channel'' mode. Transporter-mediated depolarizations associated with GABA (Haugh-Scheidt, Malchow & Ripps, 1995) and glutamate (Villalobos & Garcia-Sancho, 1995) uptake cause increases in intracellular Ca^{2+}, likely as the result of the opening of voltage-dependent Ca^{2+} channels.

Transporters Regulate pH

Acid-base changes regulate a number of cellular events and so it is intriguing that transporters participate in the regulation of intracellular pH (Bouvier et al., 1992; Rose & Ransom, 1996; Cao, Mager & Lester, 1997; Sonders et al., 1997). For some glutamate transporters, stoichiometric substrate flux likely includes H^+ or OH^- movement, while the ''leak channel'' mode of GABA and amine transporters likely includes an inwardly moving H^+.

Three different experimental systems demonstrate intracellular acidification due to glutamate transporter function. In salamander retinal glial cells, both extracellular and intracellular pH measurements show that L-glutamate and D-aspartate cause intracellular acidification (Bouvier et al., 1992). The response is Na^+-dependent and exhibits the expected pharmacology for a process involving the glutamate transporter. Similarly, fluorescence ratio imaging experiments in rat hippocampal astrocyte cultures show that glutamate and L-aspartate evoke intracellular acidification primarily due to the movement of acid equivalents associated with transporter function (Rose & Ransom, 1996). These pH changes are not restricted to glial cells; CA1 pyramidal cell apical dendrites also exhibit glutamate transporter-mediated intracellular acidification (Amato, Ballerini, & Attwell, 1994).

Bouvier and colleagues (1992) point out the potential relevance of pH regulation to situations in which neuronal activity is enhanced (e.g., stroke or epilepsy). Indeed, under ischemic conditions glial cells strongly acidify. However, OH^- flux through glutamate transporters is not the only method of transporter-mediated acidification during ischemic conditions. At low extracellular pH (<6.5), H^+ ions contribute to an inward substrate-independent leak current (Cao, Mager & Lester, 1997; Sonders et al., 1997). H^+ permeation at acidic pH appears to be a common feature of many Na^+-coupled transporters (Cao, Mager & Lester, 1997).

Transporters Regulate Extracellular Neurotransmitter Levels by Uptake and Efflux

Mechanisms

Ambient levels of neurotransmitter in the synaptic cleft will principally be determined by the interplay among neurotransmitter release, diffusion, and transporter function. The extent to which transporters participate in this process is determined in part by transport rates (*as discussed above*), the Michaelis constant for the transporter (K_d), and the density and location of these transporters at or near the synapse. Data are few regarding this latter point (but *see* Nirenberg et al., 1997). The Michaelis constants for most neurotransmitter transporters are in the high nanomolar to low micromolar range (*see* Rudnick & Clark, 1993), suggesting that transporters are capable of maintaining sub-micromolar extracellular transmitter levels independent of other processes.

Additionally, transporters can operate in reverse (*see* Attwell, Barbour & Szatkowski, 1993), and nonvesicular efflux of transmitter will contribute to ambient extracellular transmitter levels. Such reverse transport is determined by the transmembrane concentration gradients for substrates and cotransported ions, and likely occurs at more depolarized membrane potentials. Transporter-mediated substrate efflux has been hypothesized to play a role in conditions related to chronic depolarization, such as epilepsy (During, Ryder & Spencer, 1995) and excitotoxicity (Attwell, Barbour & Szatkowski, 1993; Kanai et al., 1995). Data reveal that Ca^{2+}-independent GABA efflux is a principal mode of neurotransmitter release in toad and catfish horizontal cells (Schwartz, 1987). Such efflux may also contribute to normal synaptic function mediated by other transmitters (Attwell, Barbour & Szatkowski, 1993).

Effects

In addition to direct efflux-mediated synaptic signaling, what processes will be influenced by the regulation of extracellular transmitter levels? First, synaptic and perisynaptic transmitter levels may regulate expression of neurotransmitter receptors. In a dopamine-transporter knockout mouse, both mRNA and protein levels for D1 and D2 dopamine receptors are decreased (Giros et al., 1996). Second, transporter action may regulate the amount of transmitter ''spill-over'' to more distant sites. In the hippocampus, pharmacological blockade of GABA transporters produces $GABA_B$-mediated presynaptic effects in both an autocrine (acting on its own presynaptic terminal) and paracrine manner (acting on adjacent presynaptic terminals) (Isaacson, Solis & Nicoll, 1993). Third, ambient levels of extracellular transmitter contribute to chronic low levels of receptor stimulation that occur in normal synaptic signaling (Zorumski, Mennerick & Que, 1996).

Transporter Function is Regulated through Multiple Pathways

The above data demonstrate the importance of neurotransmitter transporters in the regulation of cell signaling. Factors which modulate transporter function will necessarily influence this regulation. Below we review what is known about the factors that modulate transporter function, the triggers that initiate this process, and

the mechanisms through which this regulation is mediated.

Modulators

Kinases

A common approach to studying neurotransmitter transporter regulation has been to examine function (i.e., uptake) in endogenous and heterologous systems during treatment with various activators and inhibitors of kinases and phosphatases. For almost every neurotransmitter transporter studied, the most consistent finding is that activators of PKC and agents that maintain phosphorylated states modulate uptake, although the direction of the modulation is cell type- and transporter subtype-dependent: (i) In mammalian cells, agents that promote PKC activity reduce transport for members of the subfamily of Na^+/Cl^--dependent carriers (for examples, *see* Sato et al., 1995; Huff et al., 1997; Qian et al., 1997). (ii) In oocytes, there are multiple PKC effects (*see* e.g., Corey et al., 1994*b*; Loo et al., 1996; Zhu et al., 1997); the downregulation of function seen in some situations may be secondary to PKC-induced depolarization of the oocyte's resting membrane potential (Quick et al., 1997). (iii) For the glutamate transporter subtype GLT1 expressed in HeLa cells, PKC upregulates transport (Casado et al., 1993); for the GLAST1 subtype expressed in HEK-293 cells, PKC downregulates transport (Conradt & Stoffel, 1997).

Processes involving cAMP and calmodulin also regulate transporter function, although to date such effects have been shown only for particular transporters or in particular systems. Activators of cAMP upregulate 5HT transport in human placental choriocarcinoma cells (Ramamoorthy et al., 1993) and upregulate glutamate uptake in cultured astroglia (Gegalashvili et al., 1996). Treatment of human placental choriocarcinoma cells with specific inhibitors of calmodulin decrease 5HT transport, an effect which likely occurs independently of the cAMP-mediated effects (Jayanthi et al., 1994). For the majority of experiments involving kinase and phosphatase manipulation, the magnitude of the functional modulation is two- to fourfold. This amount of change may not appear too dramatic when the slow rates of transport are considered. However, if such changes are predominantly due to alterations in transporter number (*see below*), then this amount of modulation could exert a considerable influence on the role of the transporter in altering membrane potential or in acting as a diffusion sink.

Arachidonic Acid

Arachidonic acid (AA) is a second messenger released following phospholipase A_2 production. In salamander retinal glial cells, AA inhibits glutamate-evoked transporter currents in a dose-dependent manner (Barbour et al., 1989), and could contribute to AA's action on modulating synaptic transmission. The AA effect is glutamate transporter subtype dependent: For subtype EAAT1, AA decreases transport by reducing the maximum velocity of transport; for EAAT2, AA increases transport by increasing glutamate affinity (Zerangue et al., 1995). The actions of AA are not limited to glutamate transporters; both dopamine (Zhang & Reith, 1996) and GABA (Trotti et al., 1995) transporters are inhibited by AA. Reconstitution experiments using purified glutamate transporters suggest that AA acts through the water phase and not by altering the phospholipid membrane (Trotti et al., 1995).

pH

As discussed above, certain transporters can regulate intracellular pH through the flux of pH-changing ions; additionally, pH can also regulate transporter function. In retinal glial cells, increases in extracellular H^+ inhibit both glutamate uptake and efflux (Billups & Attwell, 1996). The authors suggest that such pH-mediated decreases in transporter function would be important in preventing the neurotoxicity that would otherwise result from glutamate efflux due to ischemia-related alterations in ion gradients. This pH-mediated decrease in transport occurs concomitantly with a decrease in the affinity of the transporter for Na^+, suggesting that H^+ ions are competing with Na^+ ions for binding sites on the transporter. Acidified extracellular pH also regulates the rat 5HT transporter expressed in oocytes (Cao, Mager & Lester, 1997). Increasing acidification results in both an inhibition of the 5HT-independent transient ''leak'' current and an enhancement of the 5HT induced ''ion channel'' current. Interestingly, the enhancement of the ''ion channel'' current is not associated with changes in 5HT uptake.

Triggers

Receptor-Mediated

Although second messenger pathways provide one route to transporter modulation, the physiological signals that initiate these second messenger changes are unknown; indeed there are few data regarding the necessary molecular ''triggers'' for regulation of transport. For AA modulation, activation of glutamate receptors on glia and neurons stimulates phospholipase A_2. Thus, receptor-mediated detection of released transmitter serves as the trigger. Such may be the case for kinase modulation as well. In rat basophilic leukemia cells, the maximum velocity of transport of 5HT is increased upon activation of adenosine receptors (Miller & Hoffman, 1994). In-

creases in 5HT transport also occur in human platelets following stimulation of histamine receptors (Launay et al., 1994). In primary astrocyte cell cultures, twofold increases in GLAST1 glutamate transporter function occur following glutamate treatment; the effect is eliminated with antagonists to AMPA/kainate receptors (Gegelashvili et al., 1996). This latter result is consistent with a model in which increases in extracellular transmitter levels, signaled by activation of receptors, feed back to upregulate glutamate transporters that will in turn act to reduce these transmitter levels.

Transporter-Mediated

Although awaiting direct experimental evidence, another hypothesis is that a signal directly associated with transport, or the transporter, provides the ''trigger'' for regulation. The evidence that changes in pH modulate transport, coupled with the evidence that pH changes can occur through transport (Cao, Mager & Lester, 1997), lends some support for this hypothesis. Additionally, chronic treatment of brain tissue with the GABA transporter blocker tiagabine leads to alterations in GABA transporter expression (Thomsen & Suzdak, 1995); however, the possibility exists that this effect is due to spillover of GABA onto GABA receptors. A similar effect on 5HT mRNA transporter expression occurs in the dorsal raphe following chronic treatment with the 5HT-transporter inhibitor fenfluramine (Rattray et al., 1994).

Mechanisms

Direct Phosphorylation

The evidence that kinases and phosphatases modulate transporter function, coupled with the presence of multiple consensus phosphorylation sites on the transporter protein, suggests that these effects are mediated through direct transporter phosphorylation. In a cloned glutamate transporter GLT1 expressed in HeLa cells, PKC-mediated increases in transport are eliminated by replacing Ser^{113} (Casado et al., 1993). For the cloned glutamate transporter GLAST1, PKC downregulates transporter function with a concomitant increase in [^{32}P] phosphate labeling of the transporter protein (Conradt & Stoffel, 1997). A similar parallel between decreased transport and increased in vivo protein phosphorylation is seen with the rat dopamine transporter expressed in LLC-PK1 cells (Huff et al., 1997).

Protein Interactions

While direct phosphorylation of some transporters may be the process through which second messengers exert their effects, not all transporters appear to be directly phosphorylated, at least at consensus sequences (Sato et al., 1995). For example, removal of the concensus PKC sites on the GAT1 GABA transporter fails to eliminate the PKC-induced modulation in oocytes (Corey et al., 1994*b*). Additionally, modulation of the expressed transporter in oocytes only occurs at lower transporter expression levels; modulation at higher expression levels can be rescued by coexpression of total rat brain mRNA Data such as these raise the possibility that transporter modulation is occurring through associations of the carrier with other proteins.

Three different experimental approaches suggest that components of the vesicle docking and fusion apparatus regulate transporter function, at least for the rat brain GABA transporter GAT1 (Quick et al., 1997); (i) PKC modulation of GABA transport in oocytes coexpressing GAT1 and total rat brain mRNA can be eliminated by injecting antisense synaptophysin and syntaxin oligonucleotides; (ii) Injection of botulinum toxins, which inactivate these trafficking-related proteins, also eliminates PKC-mediated modulation; (iii) Coexpression of GAT1 and syntaxin 1A cRNA is sufficient to permit PKC-mediated modulation. It is also interesting to note that many transporters contain leucine heptad repeat motifs which in general have been implicated as protein-protein interaction sites. Mutagenesis of a leucine heptad repeat eliminates the ability of GAT1 to be modulated by PKC (Quick et al., 1997). These data are consistent with the emerging evidence that components of the vesicle docking and fusion apparatus regulate calcium channels at the synapse (Sheng et al., 1996).

mRNA

Many of the modulatory effects on transporters occur on a time scale of seconds to minutes. For example, kinase regulation of the GABA transporter expressed in oocytes can be elicited in 5 minutes (Corey et al., 1994*b*), and this includes the time necessary for the particular reagent to cross the cell membrane or to diffuse throughout the oocyte. However, some effects occur on the time scale of hours and are due to changes in transporter mRNA levels or to transporter protein synthesis. In human placental choriocarcinoma cells, upregulation of 5HT transport following staurosporine, forskolin, or cholera toxin treatment is paralleled by similar increases in transporter mRNA levels (Ramamoorthy et al., 1993; Ramamoorthy et al., 1995). These functional effects can be eliminated by treating the cells with inhibitors of mRNA and protein synthesis. In dorsal raphe, decreases in 5HT transporter mRNA appear following chronic treatment with 5HT transport inhibitors (Rattray et al., 1994).

Transport is Regulated through Changes in Functional Transporter Number

Results from many studies of transporter regulation are consistent with the hypothesis that neurotransmitter transport can be modulated through changes in the number of functional transporters on the cell membrane. This method of regulation is a common feature of other transporters, including the Na^+-independent glucose transporter and the cystic fibrosis transmembrane regulator (*see* Corey et al., 1994*b*). Three lines of evidence support this hypothesis. First, almost all of the data from substrate saturation experiments on kinase- and phosphatase-mediated regulation of transport show changes to the maximum velocity of transport rather than to changes in the affinity of the transporter for its ligand. These data are typically interpreted as indicative of changes in transporter number rather than of changes to unitary transporter properties.

The second line of evidence comes from studies combining functional transport measurements with immunoblot analysis of transporter protein. In human embryonic kidney cells transfected with the human 5HT transporter, PKC activation causes a decrease in both 5HT transport and in 5HT-mediated currents; there is a concomitant decrease in surface transporter number based upon surface biotinylation and transporter immunoblot experiments (Qian et al., 1997). In oocytes expressing the rat brain GABA transporter, PKC activation mediates a redistribution of transporters, as assessed by subcellular fractionation and immunoblot, between intracellular locations and the plasma membrane (Corey et al., 1994*b*). In neither case are total protein levels altered, only the available pool of functional transporters. Whether this modulation involves a classically defined regulatory pathway, or a change in the rates of transporter protein exocytosis/endocytosis, or a sequestering of transporters into a nonfunctional conformation remains to be elucidated.

The third approach that implicates changes in plasma membrane transporter density in regulation of the transporter is direct estimation of the number of functional transporters on the cell surface; this procedure is based upon the fact that each transporter contributes an increment of charge movement during a voltage jump experiment (Loo et al., 1993; Quick et al., 1997). For the GAT1 GABA transporter measured in the absence of substrate, a change in membrane voltage produces transporter-related capacitive transients that are interpretable as a movement of Na^+ ions into (and out of) their binding sites on the membrane. Importantly, these transients are specifically blocked by certain GABA transporter inhibitors. Thus, a subtraction of these charge movements in the presence and absence of the blocker yields a charge movement difference from which functional surface transporter number can be calculated. This method has been used in oocytes to more directly show that PKC and members of the vesicle docking and fusion apparatus mediate alterations in functional GABA transporters (Quick et al., 1997).

Transporter Regulation Affects Behavior and Disease States

The ability of cells to regulate transporter number is not only important for normal synaptic signaling; the improper regulation of functional transporter number may also contribute to the etiology of several neurobiological diseases. In this section, we briefly highlight studies that are consistent with this hypothesis.

Early investigations into the role of the 5HT transporter in neuropsychiatric disease reported a decrease in the number of imipramine binding sites on platelets and on synaptosomes prepared from postmortem brain tissue of depressed patients and suicide victims (Briley et al., 1980; Stanley, Virgilio & Gershon, 1982). It should be noted that these findings have been questioned based on conflicting results from binding studies using paroxetine, a 5HT transporter inhibitor with higher affinity than imipramine (*see* Marcusson & Ross, 1990 for references and discussion). Regardless, selective 5HT reuptake inhibitors are used in the treatment of depression, obsessive-compulsive disorder, anxiety disorders, eating disorders and substance abuse. Some of these data suggest that this inhibition modulates transporter number (Fuller & Wong, 1990), perhaps at the level of transcription (Lopez et al., 1994).

Perturbation of hippocampal GABA-mediated inhibition has been suggested to lead to increased neuronal excitability. Studies show that there is reduced glutamate-stimulated, calcium-independent GABA release in the affected hippocampus of temporal lobe epileptics. This decrease in transporter-mediated efflux leading to reduced inhibition and seizure is thought to be due to a decrease in the number of GABA transporters (During, Ryder & Spencer, 1995).

Increased levels of glutamate in the extracellular space leads to excitotoxic cell death; this increase is believed to be a contributing factor in human neurodegenerative diseases, hypoxic cell death, and epilepsy. Such increases in extracellular glutamate could be transporter-mediated, either through decreases in uptake or through increases in efflux. For example: (i) Decreased glutamate transporter protein levels and decreased uptake occurs in brain and spinal cord samples from patients with spontaneous amyotrophic lateral sclerosis (ALS) (Rothestein, Martin & Kuncl, 1992). (ii) Decreases in glutamate transporter subtype EAAT2 protein levels from the motor cortex of ALS patients occur without

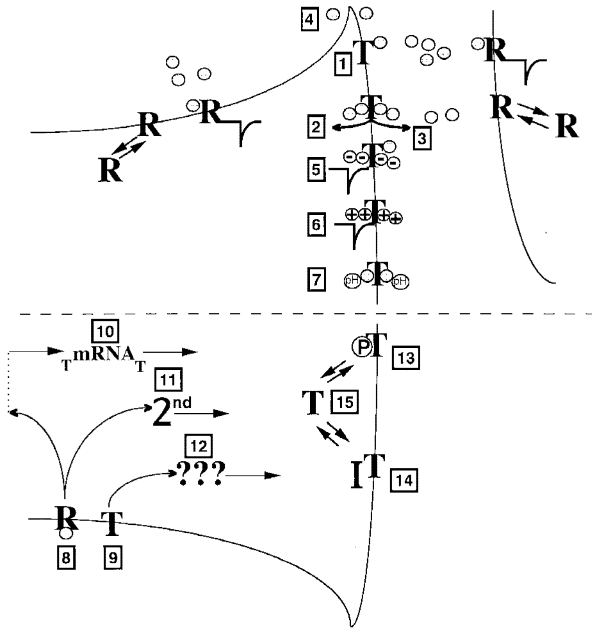

Fig. 1. A cartoon illustrating the major concepts in this review: neurotransmitter transporters regulate function (depicted above the dashed line), and are themselves subject to regulation (depicted below the dashed line). Transporters (T) can regulate cell signaling on a rapid time scale by acting as a sink for transmitters in the cleft [1]. Transporters also alter ambient extracellular neurotransmitter levels through uptake [2], and in certain circumstances, by efflux [3]. Such levels will affect both receptor (R) signaling and receptor number on neighboring cells and will serve as a mechanism for autoregulation as well [4]. Transporters regulate membrane excitability through their actions as a ''ligand-gated ion channel'' [5], and as a ''leak channel'' [6]. Transporters are also regulators of pH [7]. Potential triggers for the regulation of transporters include signals transduced through receptors [8] and the transporters themselves [9]. These signals result in long-term transporter regulation (e.g., changes in transporter mRNA levels [10]) and short-term transporter regulation [e.g., due to intracellular second messengers [11]). How transporters may trigger their own regulation is unknown at present [12]. Mechanisms of transporter regulation include direct phosphorylation [13] and interactions with other proteins [14]. Some of the regulation of transporters is due to a change in the number of functional transporters on the plasma membrane [15]. Please note that the cartoon is not implying a distinction between plasma membrane transporters on neurons or glia (e.g., Borden, 1996); nor is it implying that transporters are necessarily synaptic (e.g., *see* Nirenberg et al., 1997).

significant changes in mRNA levels and suggest modulation at the translational or post-translational level (Bristol & Rothstein, 1996). (iii) In the kindling-induced model of epilepsy, changes in protein levels for glutamate transporter subtypes GLAST and EAAT1 are seen in rat hippocampus and pyriform cortex (Miller et al., 1997). In summary, all of these studies are consistent with the idea that transporter regulation plays a role in neurobiological disease; conversely, these data also imply that the ability to regulate transporter function could be an effective strategy in the treatment of such disorders.

Conclusions

Database searches reveal an exponential growth in the number of research articles pertaining to neurotransmitter transporters, and this is particularly true for the subject of transporters and regulation. These investigations leave little doubt that transporters are principal players in both normal and abnormal cell signaling; however, even more exciting has been the discovery of a rich variety of processes through which transporters may mediate these effects. The schematic in Fig. 1 summarizes these processes. While research on transporters over the next few years will undoubtedly shed light on important questions regarding transporter regulation of cellular events (e.g., Which transporter-mediated events are physiological relevant?) and cellular regulation of transporter function (e.g., What are the signals that trigger transporter modulation?), the eventual understanding of synaptic signaling may involve asking questions at the interface of these two events; namely, how do transporters control synaptic events by regulating transporter function?

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