Neurotransmitter Transporters

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Neurotransmitters are released into the extracellular space during synaptic transmission. The actions of these chemical signals are terminated through active uptake by transporters that are located in the plasma membrane of neurons and glial cells. Transporters harness electrochemical gradients to force the movement of transmitter back into cells against its concentration gradient. These proteins play an important role in determining how long chemical signals persist, and as a result drugs that inhibit transporters produce profound behavioural effects.

Introduction

The transmission of signals between neurons occurs at specialized synaptic junctions where electrical excitability in the form of an action potential is translated into the release of a chemical messenger, or neurotransmitter, that carries the information between cells. In order for synapses to be effective at repeated signalling, neurotransmitters must be transient signals, otherwise they would accumulate in the extracellular space and activate receptors continuously. Although much of our core knowledge about synaptic transmission was obtained from studies of neuromuscular junctions, contacts between nerve terminals and muscle fibres, the mechanism used to inactivate transmitter at these structures is fundamentally different from that which occurs at most synapses in the central nervous system (CNS). At the neuromuscular junction the action of the neurotransmitter acetylcholine is terminated by acetylcholinesterase, an enzyme embedded in the matrix separating nerve terminal and muscle. This enzyme rapidly hydrolyses acetylcholine into acetate and choline, which are not able to activate receptors. At synapses between neurons in the CNS, transmitters are inactivated by efficient removal from the extracellular space. This clearance of transmitter is accomplished by high-affinity transporters, membrane proteins that shuttle transmitter across the plasma membrane and into the cytoplasm of neurons and glial cells (**Figure 1**).

Termination of Synaptic Transmission Relies on Diffusion and Uptake by Transporters

When a synaptic vesicle fuses with the presynaptic membrane, its contents of several thousand transmitter molecules are released into the synaptic cleft. The concentration of transmitter rises rapidly to several millimolar in the small volume of cleft, ensuring that transmitter molecules encounter receptors. Transmitter then dissipates through diffusion and is eventually removed from the extracellular space through uptake by transporters. At synapses throughout the CNS these two processes, diffusion and active uptake, work in concert to terminate the action of neurotransmitters. However, in addition to maintaining a low ambient concentration of transmitter so that signalling can continue, transporters can also influence synaptic signalling on a more rapid time scale.
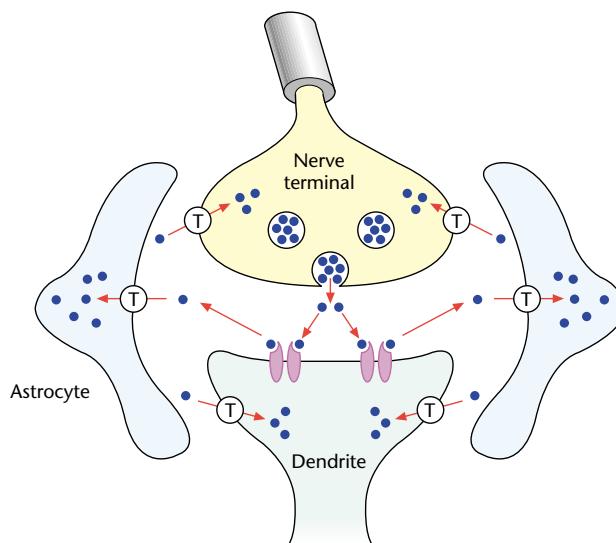

Figure 1 Neurotransmitter released during synaptic transmission is removed from the extracellular space by high-affinity transporters (T) in the membranes of neurons and surrounding glial cells (astrocytes). The action of neurotransmitters is limited by diffusion away from receptors and uptake by transporters.

The response produced when a transmitter interacts with its receptors, the synaptic potential, is the fundamental unit of communication between neurons. Whether this is a brief excitatory postsynaptic potential triggered by glutamate or a slow shift in the membrane potential produced by noradrenaline (norepinephrine), the duration of this potential determines how it interacts with other synaptic responses occurring close in time (temporal summation), and how they ultimately alter the excitability of the postsynaptic neuron. Given the critical importance of synaptic potentials in neuronal computation, it is not surprising that different neurotransmitters, and even different synapses that use the same neurotransmitter, produce responses that vary widely in their size and duration. The shape of these synaptic responses is determined by many factors, including the geometry of the synapse, the type of transmitter released, and the properties, number and location of the receptors underlying the response. Transporters can influence the shape of synaptic potentials by altering how many receptors are activated and determining for how long they are exposed to transmitter.

The role of transmitter uptake in shaping synaptic responses has been tested by perturbing uptake by applying drugs that selectively block transport, or through molecular genetic approaches, such as the generation of transgenic animals that lack certain transporters. These manipulations often have profound effects on synaptic transmission and in the whole animal often lead to drastically altered behaviours, each unique to the particular transmitter system disrupted. Although our understanding of transporter involvement in synaptic signalling is incomplete, these studies indicate that transporters can: (1) lower the peak concentration of transmitter in the synaptic cleft if they are present at a high density near the receptors, (2) decrease the time for which transmitter is available to activate receptors and (3) shorten the distance over which the transmitter diffuses after it is released. The latter function will minimize both the diffusion of transmitter to receptors at neighbouring synapses (termed 'spillover' or 'crosstalk') and the mixing of transmitter when neighbouring synapses are active at the same time (termed 'pooling').

The ability of transporters to limit the duration of synaptic potentials will depend on the rate at which transmitter diffuses away from receptors relative to the rate at which the transmitter unbinds from receptors. If transmitter diffusion is slow, then transporters can dramatically influence the time for which transmitter is available to activate receptors. Not surprisingly, the effect of transport inhibition varies considerably from one synapse to another, and at individual synapses certain receptors may be more sensitive to transport block depending on their affinity and their location relative to the site of transmitter release. The effect of transport inhibition may be more acute for transmitters such as serotonin and noradrenaline, which appear to be released from varicosities in a paracrine fashion, without clearly defined synaptic contacts. These transmitters may have to travel a greater distance to reach receptors, increasing the potential for transporters to influence receptor activation. It is interesting to note that the prolongation of some synaptic responses that is observed during transporter inhibition is paralleled by the changes in responses recorded at the neuromuscular junction when acetylcholinesterase is inhibited, suggesting that the two mechanisms for removing transmitter – uptake and enzymatic hydrolysis – are functionally quite similar.

Identification and Localization of Transporters

Initial studies using radioactively labelled transmitters localized the sites of transmitter accumulation to neurons through autoradiography. Accumulation was generally restricted to regions where the endogenous transmitter was released, suggesting a specific mechanism for uptake. Transmitter uptake was rapid and the magnitude of the uptake correlated with the density of innervation, disappearing when neuronal inputs were removed. Through these studies it became clear that there was an active uptake mechanism, presumably located in nerve terminals, that was capable of concentrating transmitter more than 10 000-fold. Recent studies using sensitive immunocytochemical methods have led to refinements in our knowledge about the location of transporters relative to the sites of transmitter release. These studies indicate that transporters are found in both neurons and glial cells, and are often excluded from synaptic membranes.

Molecular diversity of transporters

The first deoxyribonucleic acid (DNA) sequence for a neurotransmitter transporter was obtained through purification, amino acid sequencing, then cloning of the molecular species responsible for high-affinity γ-aminobutyric acid (GABA) uptake. This was soon followed by identification of the noradrenaline transporter by means of expression cloning. Based on their close sequence similarity, these two transporters comprise a large gene family which also includes transporters for the neurotransmitters serotonin, glycine and dopamine, as well as for choline, proline and taurine, collectively referred to as the 'biogenic amines'. Transporters for the excitatory amino acids, glutamate and aspartate, are members of a separate family of transporters that differ both in their structure and in the way that they accomplish uptake (see below).

There is much less diversity in transporter species than has been found for neurotransmitter receptors. For example, only a single serotonin transporter gene has been

identified (termed *SERT* for *ser*otonin *t*ransporter), but there are more than a dozen genes encoding serotonin receptors. This is also true for the transport of other monoamines, dopamine (termed DAT for *dop*amine *t*ransporter) and the norepinephrine–epinephrine (noradrenaline – adrenaline) (termed NET for *nor*epinephrine *t*ransporter), for which there is only one transporter each. These transporters are related to the H^+-dependent transporter that forces transmitter into synaptic vesicles (termed VMAT for *v*esicular *mono*amine *t*ransporter). In contrast, there is a greater diversity in the transporters for 'fast' neurotransmitters that directly gate receptor–ion channels, such as glycine, GABA and glutamate. There are two genes for the glycine transporters (termed *GlyT* for *gly*cine *t*ransporter), four GABA transporter genes (termed *GAT* for *GA*BA *t*ransporter) and five genes encoding glutamate transporters (termed *EAAT* for *e*xcitatory *a*mino *a*cid *t*ransporter). EAATs are closely related to the transporters of neutral amino acids (termed ASCT for *a*lanine, *s*erine, *c*ysteine *t*ransporters), dicarboxylate, as well as the bacterial glutamate transporters. Within each family, members share approximately 50% homology with one another. In some cases, different transporter isoforms are formed through alternative splicing of the respective messenger ribonucleic acids (mRNAs), but this is also not as prevalent as that observed for transmitter receptors.

Transporters are found in neurons and glial cells

The localization of these different transporters has been investigated systematically using subtype-selective antibodies. These immunocytochemical studies have reinforced some of the conclusions obtained through autoradiography, namely that transporters for a particular neurotransmitter are present in the plasma membranes of the neurons that synthesize and release the same neurotransmitter. For example, noradrenaline transporters are found in the noradrenergic neurons of the locus ceruleus, serotonin transporters are found in neurons of the raphe nuclei, and GABA transporters are found in the membranes of inhibitory neurons throughout the CNS. These studies also revealed that transporters for essentially every neurotransmitter are expressed by glial cells, in particular astrocytes and astrocyte-like cells, such as the Bergmann glial cells of the cerebellum and the Müller cells of the retina. For transporters that have multiple subtypes, one or more of the transporters may have an exclusive glial localization. For instance, EAAT-1 and EAAT-2 glutamate transporters, GAT-3 GABA transporters, and GlyT-2 glycine transporters are all essentially restricted to glial cells. Certain transporters have highly restricted distributions, such as EAAT-4, which is found only in cerebellar Purkinje neurons, whereas others are widely expressed,

such as GAT-2, which is found in neurons, astrocytes and meningeal cells throughout the CNS. Although knowledge about the distribution of transporters provides clues about their function, the particular contribution of each subtype in transmitter reuptake is poorly understood.

Why are transporters expressed by astrocytes? Astrocytes are interspersed between neurons, and in many instances their processes wrap around, or 'ensheath', synapses. The intimate relationship between astrocyte processes and synapses may limit interactions between neighbouring synapses, by increasing the distance over which a neurotransmitter must travel to encounter receptors at a nearby synapse. By providing an additional sink for neurotransmitter through the expression of transporters, astrocytes decrease the distance for which a neurotransmitter travels after it is released. Thus, astrocyte transporters may help to preserve the autonomy of individual synapses, despite their high density within the neuropil.

Transporters are localized to perisynaptic regions

The combination of immunocytochemistry and electron microscopy has provided unprecedented resolution of the distribution of transporters. By labelling thin sections of brain tissue with transporter specific antibodies attached to small gold particles, it has been possible to visualize the cellular and subcellular distribution of transporters in cell membranes with an electron microscope. These studies have forced a revision of the preconception that neuronal transporters are localized to the presynaptic membrane. Instead, transporters are often found along axons, cell bodies and dendrites. In most cases, transporters appear to be excluded from the region of membrane within the synaptic cleft, with the highest density occurring in the perisynaptic membrane, just outside the synapse in either the axonal or dendritic membrane. The highest density of transporters in astrocyte membranes is found nearest to synapses. Neuronal and glial transporters thus appear to form a gauntlet that transmitter must pass through in order to reach receptors at neighbouring synapses. The exclusion of transporters from the synaptic cleft may limit their ability to compete with receptors for binding of transmitter.

Transport is Coupled to Ion Translocation

In initial studies of living sections of brain or in isolated membrane preparations, accumulation of radiolabelled transmitter occurred with high affinity, was highly temperature dependent, required energy, and could be

saturated, with no further increase in the rate of uptake observed above a certain concentration of transmitter. These results suggested that this rapid form of transmitter uptake occurred through a receptor-mediated process rather than through unidirectional diffusion into cells. A clue to deciphering the mechanism of uptake came when researchers discovered that uptake was dependent on external Na^+ and adenosine triphosphate (ATP) levels, but was blocked when the Na^+/K^+ ATPase (the sodium–potassium pump) was inhibited, suggesting that uptake was dependent on the electrochemical gradient established by the ATPase. We now know that these Na^+-dependent neurotransmitter transporters act as symporters, using the energy stored in the unequal distribution of ions across the plasma membrane to move transmitter against its concentration gradient.

Transporter stoichiometry

The two families of transporters use different ion gradients to accomplish transmitter uptake. While both are dependent on external Na^+ concentration, transporters for the monoamines, GABA and glycine are also coupled to the movement of Cl^- into the cell, while excitatory amino acid transporters are dependent on external H^+ and internal K^+ levels, but not that of Cl^-. The movement of ions and transmitter during uptake occurs in a defined ratio, with one to several ions coupled to the movement of one molecule of transmitter. All transporters transport only one molecule of transmitter per cycle.

The particular ratio of ions and substrate transported, the stoichiometry, is determined by measuring how transmitter accumulation, or flux, varies with changes in the concentration of a particular ion. Fitting this relationship with the Hill equation yields a coefficient representing the number of ions cotransported with each molecule of transmitter. A first-order dependence of flux on the concentration of a particular ion indicates that one ion is translocated per molecule of transmitter; a second-order dependence indicates two ions are translocated per molecule of transmitter; and so on. Currently, the precise stoichiometry has been determined only for some transporters. For example, the glycine transporter GlyT-2 cotransports $3Na^+$ and $1Cl^-$ with each molecule of glycine, whereas the glutamate transporter EAAT-3 cotransports $3Na^+$ and $1H^+$ with each negatively charged molecule of glutamate, and countertransports $1K^+$. Because of the unbalanced movement of charges, the transport of each molecule of transmitter results in the net movement of one to three positive charges into the cell, depending on the stoichiometry of the transporter. This electrogenic property of transporters has greatly aided studies of transporter behaviour, as uptake can be monitored using sensitive electrophysiological methods.

The concentration gradient that a particular transporter can achieve at equilibrium is determined by the stoichiometry of transport and by the concentrations of the ions and substrate that are coupled to transmitter flux. For example, EAAT-3 glutamate transporters can lower the concentration of glutamate outside the cell to:

$$[glu]_{out} = [glu]_{in} ([Na^+]_{in}/[Na^+]_{out})^3 ([H^+]_{in}/$$

$$[H^+]_{out}) ([K^+]_{out}/[K^+]_{in}) \exp\{(2)VF/RT\}$$

where V is the membrane potential, R is the gas constant, T is the temperature and F is the Faraday constant. By coupling the movement of transmitter with several ions, transmitter can be accumulated against a larger concentration gradient. This mechanism ensures that cells are still capable of taking up glutamate, despite having a high intracellular concentration of glutamate. The effectiveness of the uptake process is remarkable, as EAAT-3 transporters are capable of establishing a concentration gradient across the cell membrane of greater than 10^6 at equilibrium under physiological conditions. However, the concentration of transmitter in the extracellular space is not maintained at the level predicted by these equilibrium measurements; rather, it fluctuates with neuronal activity, varying over time and with distance from sites of release.

A model for transport

The ability of transporters to bind ions and transmitter from the outside, release them to the inside, and return back to the outside for another cycle of transport, led to early models of transporters that moved across the membrane or rotated in place. The structural information contained in the primary sequence of the transporter genes and a wealth of experimental data now indicate that transporters form a pore through the membrane, reminiscent of ion channels. The movement of ions and substrates into and out of the pore is controlled by two 'gates' – one to the outside and one to the inside – that open at alternate times (**Figure 2**). According to this 'alternating access' model, the sequential binding and unbinding of ions and transmitter induce conformational changes in the transporter necessary to the open and close the gates. When the outer gate is open, Na^+ binds, forcing the transporter into a conformation that will accept transmitter (**Figure 2a**). When transmitter binds, the outer gate closes (**Figure 2b**) and, with some delay, the inner gate opens. Upon opening of the inner gate, the ions and substrate unbind and the gate closes (with or without the binding of another ion) (**Figure 2c**). Again with some delay, the outer gate opens and, if necessary, releases ions to the outside (**Figure 2d**), revealing the binding sites for Na^+ and substrate, and allowing the transport cycle to begin again. In some conditions ions can pass through transporters uncoupled from the movement of transmitter. This leak of ions
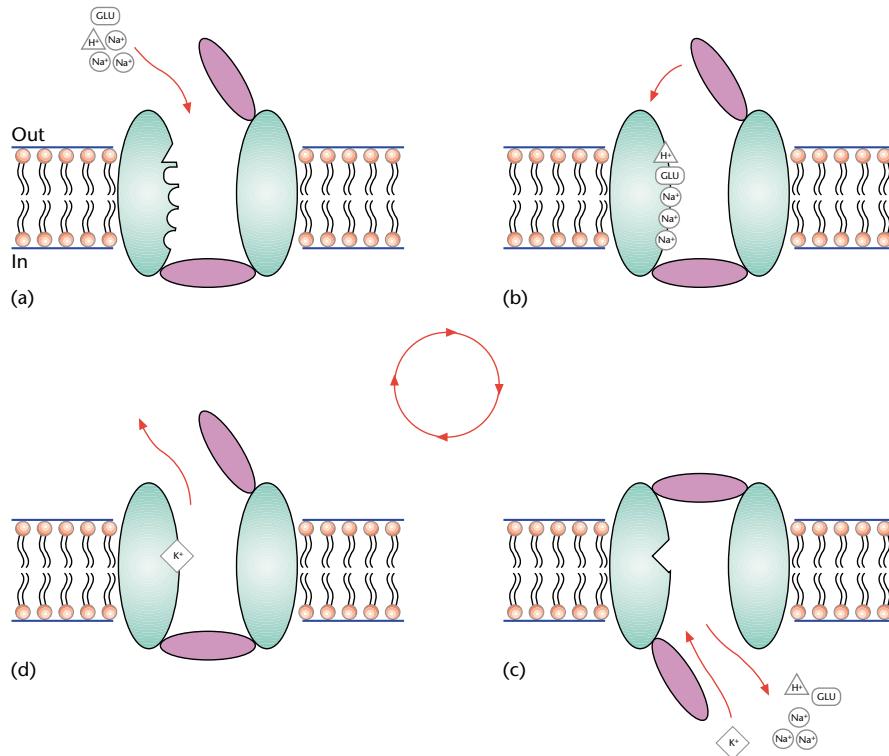
Figure 2 Alternating access model for transport of the neurotransmitter glutamate. (a) When the outer gate is open Na$^+$, glutamate (GLU) and an H$^+$ bind within the pore. (b) The outer gate closes. (c) With some delay the inner gate opens, allowing Na$^+$, glutamate and an H$^+$ to dissociate. K$^+$ then binds within the pore, closing the inner gate. (d) With some delay the outer gate opens, allowing K$^+$ to unbind and revealing the binding sites for Na$^+$, glutamate and an H$^+$, starting the cycle over again.

through the transporter is thought to occur during moments when the two gates are open simultaneously.

Neurotransmitter transporters require tens to hundreds of milliseconds to complete one cycle of transport, reflecting the time necessary for the sequential binding and unbinding of ions and substrate, and for the conformational changes necessary to ensure translocation. With the net movement of only one or two charges per cycle, the rate of ion flux is many orders of magnitude slower than through ion channels (1–40 per second for transporters versus 10^6 per second for ion channels). For this reason uptake rarely induces significant changes in the membrane potential of cells. How do transporters accomplish rapid clearing of transmitter from the extracellular space with such a slow turnover rate? At the neuromuscular junction, acetylcholinesterase rapidly inactivates acetylcholine at a rate of 10^4 molecules per second. Transporters compensate for their slow turnover rate by being expressed at high densities, often thousands per square micron, and by having a high affinity for neurotransmitter, ranging from less than one micromolar for serotonin transporters to tens of micromolar for most of the glutamate transporters. This yields a transporter population with a high capacity for transmitter uptake that

is not likely to be saturated by the repeated release of the several thousand transmitter molecules present in each synaptic vesicle.

In principle, transport is not unidirectional, and reversed transport can expel transmitter into the extracellular space. Reversed transport may be used by some cells to release transmitter when conventional vesicular release mechanisms are not present: horizontal cells in the retina release GABA though reversed transport, and astrocytes may release glycine through reversed transport. In addition, reversed transport becomes more likely in pathological conditions such as brain ischaemia when intracellular ATP levels drop and ion gradients collapse. In this way, reversed transport of glutamate may exacerbate brain injury by causing the sustained activation of neuronal glutamate receptors.

Structure of Neurotransmitter Transporters

The initial cloning and subsequent determination of the primary sequence of several of the biogenic amine

transporters revealed that they are glycoproteins consisting of approximately 600 amino acids, with a molecular weight of between 60 and 85 kDa. Hydropathy analysis, which predicts the arrangement of transmembrane segments of a protein based on the relative locations of hydrophobic and hydrophilic residues, suggests that they consist of 12 membrane-spanning α helices, with a large glycosylated extracellular loop between the predicted third and fourth transmembrane domains. As expected from their high degree of sequence similarity, all members of this family share a similar structure. The membrane topologies of the excitatory amino acid transporters have been more difficult to predict based on their sequences. While there is a consensus that the N-terminus of the protein contains six membrane-spanning regions, the topology of the region near the C-terminal end has been more difficult to determine. It has been proposed to cross the membrane from zero to four times. Recent modelling suggests that a portion of this region forms a 're-entrant loop', reminiscent of the structure of the pore region of ion channels. Further clarification of the structure of these proteins will be obtained through crystallization and X-ray diffraction studies.

The lack of a signal sequence at the N-terminus suggests that both the N- and C-termini of the transporters face the cytoplasm. These intracellular segments may be important for regulating the targeting of transporters to specific regions of the cell membrane, or their activity through interaction with other cytoplasmic proteins. These segments contain several serine and threonine residues, suggesting that transport may be modulated by phosphorylation. Stimulation of kinases within cells can alter transporter activity by changing either the number of functional transporters at the cell membrane, their maximal transport capacity (V_{max}), or their affinity (K_m). These intracellular regions may not only be sites for phosphorylation, but may also provide a link between transporters and other signalling complexes, or even other transporters. Although transport can be induced in expression systems by a single RNA species, it is not known whether transporters function singly or together as a complex of several transporters. Oligomer bands are often seen on sodium dodecyl sulfate–polyacrylamide gels of transporter proteins, suggesting that transporters may exist in the brain as multimeric units connected via noncovalent interactions.

Transporters as Targets for Drugs

By clearing transmitter from the extracellular space after release at synapses, high-affinity transporters shape the spatial and temporal activation of receptors that is critical for appropriate signalling in neural circuits. As a result, drugs that inhibit these transporters produce profound behavioural effects. Many antidepressant, antihypertensive and psychostimulant drugs, including well-known addictive and abused compounds, inhibit monoamine transporters. Perhaps the most notorious of these, cocaine, exerts its euphoric effects through inhibition of DAT transporters on dopamine neurons that are involved in reward pathways. Although cocaine is a relatively potent antagonist of all three monoamine transporters, the importance of DAT inhibition for producing the psychostimulant effects of cocaine was shown by generating transgenic mice that lack DAT transporters. These mice exhibited the same hyperactivity seen in normal mice after cocaine administration, and showed only physiological responses to cocaine that could be attributed to the inhibition of other monoamine transporters.

Tricyclic antidepressant drugs also inhibit monoamine transporters, and have become powerful therapeutic tools for treating numerous psychiatric illnesses such as mental depression, eating disorders (obesity, bulimia), obsessive–compulsive disorders and panic disorders. Perhaps the best known member of this class of compounds is Prozac (fluoxetine), a serotonin-selective reuptake inhibitor. Despite the ability of these drugs to inhibit monoamine reuptake, the precise role of transporter inhibition in generating the observed behavioural modifications is still uncertain, because the therapeutic effects of antidepressant drugs are not observed until they have been administered continuously for several weeks. Furthermore, many psychotropic drugs have other sites of action due to their structural similarity to neurotransmitters. For example, the stimulant amphetamine, in addition to inhibiting the uptake of noradrenaline and dopamine, inhibits VMAT, and thus the loading of transmitter into vesicles, and is also a partial agonist at noradrenaline receptors. In addition, some inhibitors are also substrates for the transporters, and can cause the release of transmitter from cells through a process called heteroexchange. A clearer picture of the role of these compounds will emerge as more selective transport inhibitors are developed and we obtain a more complete understanding of the neural pathways involved in generating various behaviours.

Transporters for the excitatory and inhibitory neurotransmitters, glutamate and GABA respectively, have also been targeted to ameliorate some human neurological diseases. GABA transport inhibitors such as tiagabine have powerful anticonvulsant and antiseizure properties, and are effective in treating some epilepsies. These compounds decrease the excitability of neurons through persistent activation of inhibitory GABA receptors on neurons. Conversely, experimental disruption of glutamate uptake in animals induces seizures, and a loss of EAAT-2 transporters may contribute to the death of motor neurons that occurs in amyotrophic lateral sclerosis (Lou Gehrig disease) through overstimulation of glutamate receptors on these neurons (excitotoxicity). There is hope that increasing the number or activity of glutamate

transporters may help limit excitotoxicity by speeding up clearance of glutamate released in these abnormal conditions. However, transporters have the ability to accumulate transmitter-like molecules that are neurotoxic, raising awareness that transporters may be an important link between endogenous or environmental toxins and certain neurodegenerative diseases such as Parkinson disease.

Summary

The transmission of signals between neurons at synapses relies on the release of a chemical neurotransmitter. To ensure that these signals do not persist indefinitely, transmitter action is stopped by uptake back into cells. This uptake is achieved by high-affinity transporters located in the plasma membranes of neurons and glial cells. Transporters use the energy stored in the electro-chemical gradients for Na^+ and other ions to move transmitter against its concentration gradient into the cell. Transport is an ordered process whereby the sequential binding and unbinding of ions is coupled to the translocation of a single molecule of transmitter during each cycle of transport. Drugs that inhibit neurotransmitter transporters prolong the actions of transmitters, and comprise both drugs of abuse and those with tremendous therapeutic potential for the treatment of various neurological disorders.

Further Reading

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