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# Transport of Receptors

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## Abstract

The axonal transport of neurotransmitter receptors is thought to be a common phenomenon in many neuronal systems. The "machinery" for receptor (protein) "assembly" is found in the cell bodies of neurons and the "manufacture" of receptors takes place there. These receptors are then "shipped" to their ultimate destinations by a transport process. This is an axonal transport mechanism in the case of presynaptic receptors. Some form of transport process may also exist to send receptors out into the dendritic arborizations of neurons, although the latter is more difficult to verify. Axonal transport has been demonstrated, in the peripheral nervous system, for many different neurotransmitter receptors. In the central nervous system, the results are less clear, but indicate the presence of a transport mechanism for catecholamine, acetylcholine, and opiate sites. One important component then, in the development of receptors, is the transportation to terminal membrane sites where they are ultimately incorporated and available for interaction with neurotransmitters and drugs.

**Index Entries:** Presynaptic receptor; axonal transport of receptors; receptor flow; heteroreceptors; muscarinic receptors; opiate receptor; β-adrenergic receptor.

## Introduction

Evidence exists for the transport of receptors in axons of peripheral nerves (Zarbin et al., 1982; Laduron and Castel, 1990). Many of these studies utilize axonal ligation or compression (crush) techniques that were originally developed for the study of transport of neurotransmitters (Dahlström, 1965). The ability to bind radioactive ligands to receptors in vitro, using appropriate drugs and conditions established in membrane-binding studies, coupled with autoradiographic localization (Kuhar et al., 1986) of these sites in ligated neurons, made it possible to establish that axonal transport of receptors was taking place. The predominate difficulty encountered involved the ability to identify tracks of axons that were easily accessible for such ligation experiments. This was much easier to perform in the peripheral nervous system. Other methods had to be developed to establish the existence of axonal transport mechanisms in the brain.

There is evidence for both anterograde (from the cell body to the axon terminal) and retrograde (from the axonal terminal back to the cell body) transport of receptors. Intuitively, in any situation in which receptors are located on terminals, such as in the axon, these receptors must be moved from the cell body to that position. Receptors are peptidergic entities and the apparatus for protein synthesis exists in the cell body. Some form of transport must occur in order for the receptors to reach the terminals.

Anatomical descriptions of specialized synapses (Somogyi, 1982,1983) have referred to the presence of axoaxonic synapses (one axon bouton ending on another axon's terminal). Physiological studies have indicated the presence of presynaptic receptors that limit the release of neurotransmitters from the neuron (Chesselet, 1984; Laduron, 1985). When one neurotransmitter is affecting the release of a completely different neurotransmitter, its effects are mediated via heteroreceptors. For example, the ability of dopamine to enhance GABA release represents the presence of dopamine heteroreceptors on GABAergic terminals in the substantia nigra-pars reticulata (SNR: Reubi et al., 1977). Likewise, dopamine is capable of reducing the firing rate of dopaminergic neurons in the substantia nigra-pars compacta (SNC). These receptors are known as autoreceptors since they mediate effects of the neurotransmitter released from that same particular type of neuron (Bunney and Aghajanian, 1973; Nissbrant et al., 1985).

Molecular biological studies have indicated the presence of receptor subtypes based on identification of the genetic code responsible for the synthesis of each subtype. For instance, in the dopamine system, there are at least five receptor subtypes that have been isolated and cloned (Sokoloff et al., 1990; Tiberi et al., 1991; Van Tol et al., 1991). In the muscarinic cholinergic system, the sequence of DNA for the synthesis of five distinct subtypes has also been elucidated (Bonner 1989; Liao et al., 1989). The genetic information can be duplicated so that complementary DNA (cDNA) probes are formed allowing the use of techniques, such as *in situ* hybridization, to identify the messenger RNA (mRNA) associated with expressing each of the various receptor subtypes (Lewis et al., 1985). The mRNA associated with receptor synthesis (and all mRNA for that matter), has been localized in cell bodies. Thus, the verification that synthesis of these receptors occurs in the cell body is now apparent.

Difficulties in demonstrating the shipping procedure relate to the paucity of receptors that are undergoing transport at any one period of time. Thus, it is necessary to cause these receptors to accumulate over time in order to show their appearance in axonal membranes. Once present in the terminal membrane, the presynaptic receptors are thought to mediate an inhibition process (Starke, 1981; Kupferman, 1979). Stimulation of the terminal receptors sets into play a cascade of events that may involve various second messengers or a more direct effect on ion channels. If action potentials are subsequently conducted to the terminal area, a reduction in calcium influx will result, not as many synaptic vesicles will combine with the membrane, and not as much

neurotransmitter will ultimately be released. In this way, this presynaptic mechanism has reduced the effects on the postsynaptic cell. This type of presynaptic inhibitory mechanism has been hypothesized to exist in many neuronal systems. It also may be a very important field for the development of new pharmacologic agents.

## Retrograde Transport

Retrograde transport is presumably necessary in order to degradate the neurotransmitter receptors. Retrogradely transported receptors do not accumulate to the same extent as those transported in the anterograde direction (Young et al., 1980; Wamsley et al., 1981; Laduron and Janssen, 1982; Zarbin et al., 1981,1982,1983,1990). One would assume there would be an overall accumulation of receptors at the terminal since import does not equal export. This could be explained, however, by the presence of packaging of membrane components that are shipped together back to the cell body for degradation by the lysosomal organelles. Not all of these receptors may be available for binding, and there may be an affinity difference between the receptors undergoing retrograde as opposed to anterograde transport. Interestingly, receptors that are undergoing anterograde transport appear to be in the high-affinity conformation (Zarbin et al., 1982,1983). As certain receptor populations are synthesized in the cell body, they may be coupled to a guanine nucleotide regulatory protein (G-protein). The presence of this G-protein, that is unoccupied by a nucleotide, keeps the receptor in its high affinity conformation until it reaches the terminal where high concentrations of guanine nucleotide are present (Rodbell, 1980; Dolphin, 1987). At this time, the catalytic enzyme is coupled to the complex, the regulatory protein is occupied, and the receptor is switched to a low affinity conformation. This would appear to be the time when the receptors are incorporated in the membrane and become functional.

## Transport of Uptake Sites

Other phenomena may require the presence of presynaptic receptors which may be identified as transported sites. A rigid physiological definition of the term "receptor" necessitates that a biological consequence occur when these sites are occupied by a neurotransmitter. This may not always be the case, owing to the existence of "spare" receptors and uptake sites, among others. The term "receptor" is used loosely in this context and perhaps could more appropriately be replaced with the terms "binding" or "recognition" sites. This is important since, for example, it is known that a potent and efficacious reuptake system is involved as the principal means of removing many neurotransmitters from the synaptic cleft. This is necessary to clean out the system rapidly so that the neuronal interaction is renewed and capable of responding to the next volley of impulses. There must be some way for the presynaptic cell to recognize the presence of the neurotransmitter and to bind it for endocytotic vesiculation and recovery. These "receptors" need not be functional *per se* (Raiteri et al., 1984), but rather are necessary for recognition (binding) and subsequent removal of the neurotransmitter. This appears to be a very specific process since only the neurotransmitter released by a particular neuronal population is resequestered by that group of neurons. This principle has been exploited by neuroanatomists as a retrograde tracing technique to identify specific neurotransmitter-containing pathways (Cowan and Cuenod, 1975). Colocalization of neuropeptides and neurotransmitters, both of which may be subject to a reuptake process, may make interpretation of the results of this technique a bit more difficult. Identification of the particular neurotransmitter released independent of others must involve some type of recognition sites in these uptake systems. Other binding sites associated with ion channels responding to ouabain and tetrodotoxin have also been shown to be involved in an active transport process (Lombet et al., 1985,1986).

## Rate of Receptor Transport

Transport of receptors is thought to take place by the process of fast axonal transport since the mechanism appears to be disrupted by colchicine. Microtubular formation (a characteristic of fast axonal transport) is thus apparently necessary for the transport of receptors to take place (Schwartz, 1979; Grafstein and Forman, 1980). However, attempts to measure the actual rate of transport indicates an intermediate rate of approximately 10 mm/day (Zarbin et al., 1982,1983,1990). These measurements rely on several assumptions, some of which are known to be oversimplifications. Not all of the receptors are thought to be moving at any one instant in time and interruption of flow of the axonal constituents, either by nerve crush or ligation, undoubtedly causes local reflux and membrane changes in the immediate vicinity of the cellular damage (Bisby, 1987). A more sophisticated method for determining the rate of transport will need to be applied before an accurate rate of axonal transport of receptors can be determined.

Peak accumulations of receptors appears to occur between 12 and 24 hours. This could reflect the limit of viability of the axon separated from the cell body, rather than indicating any peak elevation based on the transport mechanism. Double ligation experiments demonstrate that connection with the cell body is not a prerequisite for the axonal transport of receptors to take place. Accumulation occurs proximal to both the initial and isolated segments. Retrograde transport is characterized by similar accumulations distal to the two ligatures.

## Receptor Transport in the Vagus Nerve

Axonal transport of receptors in ligated vagus nerve preparations has been the most frequently studied. The original report involved evidence of axoplasmic transport of opioid receptors (Young et al., 1980). Initial investigations did not show much evidence for the presence of retrogradely transported receptor sites (Young et al., 1980). This was not the case in the study of opioid receptors labeled in vivo (Laduron and Janssen, 1982) or transported muscarinic cholinergic receptors labeled in vitro (Laduron, 1980; Zarbin et al., 1982). Other reports of transported receptors in the vagus nerve include NMDA (glutamate), cholecystokinin, neurotensin, angiotensin II (Cincotta et al., 1989; Zarbin et al., 1981; Kessler and Beaudet, 1989; Diz and Ferrario, 1988), and probably a few others.

In vitro techniques of autoradiography, which were established for localization of drug and neurotransmitter receptors, were applied to this dynamic system by looking at receptor accumulations over time. Animals were anesthetized and the vagus nerve exposed and ligated in such a fashion so as to interrupt transport along the axons present in the bundle. By providing the animal with a postligation survival period, the transported receptors were allowed to accumulate. The nerve was subsequently removed, sectioned longitudinally in a cryostat, and labeled in vitro with a radioactive compound known to be specific for the receptor population of interest, using predefined conditions. The buildup of receptors in the constituent neuronal populations could then be examined after generation of autoradiograms. Analysis of the autoradiograms showed the accumulated sites, bound by the radioactive drug, adjacent to the ligature.

Some sites that accumulate in the ligated vagal nerve trunk show typical characteristics of opiate receptors (Zarbin et al., 1990). They are subject to regulation by guanine nucleotides, the binding is sensitive to sodium, the receptors recognize opioid ligands stereospecifically, and the accumulation at a ligature can be eliminated by introducing colchicine into the neuronal sheath. Multiple types of opioid receptors have been shown to exist (Goodman and Pasternak, 1984) and the transported sites show a pharmacologic profile in keeping with what would be expected of mu-opioid receptor binding. The highest accu-

mulation at the ligature site was seen at 12 h. Again, little evidence of retrograde transport could be identified. Opioid receptors also appear to be transported centrally since these receptors appear to be presynaptic on vagal terminals in the brainstem (Atweh et al., 1978).

A question arises as to what possible functional significance vagal presynaptic receptors have. Several neuropeptides and neurotransmitter systems have been localized within the vagus nerve (Lundberg et al., 1987) and it has both motor and sensory components. The nodose (inferior) ganglion receives vagal nerve afferents that innervate (among other areas) the gastrointestinal (GI) system. Functionally, some neuropeptide receptors may exist on these sensory fibers and respond to gut hormones released according to constituents of the diet. For instance, the hormone CCK is released from gastric cells in response to the fat content introduced into the GI tract. The vagus nerve is known to be involved in suppression of appetite. One could envision a system where CCK is released to stimulate the gall bladder and introduce bile into the small intestine (duodenum) as a fat emulsifier. At the same time, this hormone stimulates vagal afferents that apprise the behavioral areas of brain of the high fat content and effectively suppress the appetite.

## Sciatic Nerve

Muscarinic and nicotinic cholinergic receptors have been shown to be transported in the sciatic nerve (Dahlström, 1983; Millington et al., 1985; Wamsley et al., 1981; Gulya and Kasa, 1984; Zarbin et al., 1982). These receptors accumulate over time (peak accumulation at 12–24 h) and are being propagated in both the anterograde and retrograde directions. The affinity of the muscarinic receptors appears to be regulated by the presence of a guanine nucleotide regulatory protein (Zarbin et al., 1982). Anterogradely transported sites are in the high affinity state and are responsive to the presence of guanine nucleotides. At least a portion of these receptors have been associated with the cell bodies of the sensory neurons (pseudounipolar neurons) in the dorsal root ganglia (Wamsley et al., 1981). Receptors propagated in the retrograde direction are in the low affinity conformation and are unresponsive to guanine nucleotides.

Another population of receptors transported in the sciatic nerve is the β-adrenergic receptors (Zarbin et al., 1983). These receptors accumulate over time with a peak density at 13 h. Again, receptors transported in the anterograde direction are sensitive to guanine nucleotides whereas those transported in the retrograde direction are not. These are characteristics of high versus low affinity β-receptor populations respectively. The transported sites show $\beta_2$-adrenergic receptor pharmacology and the accumulate on the proximal and distal aspects of double ligatures placed on the sciatic nerve trunk. At least part of these receptors are associated with sympathetic fibers that project through the sciatic nerve.

## Dorsal Roots of Spinal Nerves

The dorsal roots represent sensory neurons carrying information from the periphery, through the spinal nerves, into the spinal cord. The cell bodies for these neurons reside in the dorsal root ganglion. By ligating these tiny fibers, it has been possible to show the presence of axonal transport of opiate receptors (Zarbin et al., 1990). These sites appear to represent presynaptic receptors on sensory nerves that terminate in the dorsal horn. These may be the substance P-containing fibers (Laduron, 1984), that respond to pain and other modalities (A delta and C fibers), and serve to reduce transynaptic propagation of information via an opiate-mediated presynaptic inhibitory mechanism (Basbaum and Fields, 1984). Sensory afferents in the dorsal roots also appear to be transporting nicotinic cholinergic receptors (Ninovic and Hunt, 1983).Axonal transport of cholinergic receptors has also been reported in the splenic nerve of the dog (Laduron, 1980), the hypogastric nerve of the cat (Alonso et al., 1982), and in the optic nerve of the goldfish (Henley et al., 1986).

## Receptor Transport in the CNS

Since ligation or compression of axons in the brain is virtually impossible, other methods had to be devised to encourage accumulation of transported receptors. The most frequently used technique involves simple transection of the fiber pathway with the expectation that the transported sites will accumulate at the cut ends of the nerves (Snowhill and Wamsley, 1983; Dawson et al., 1985; Van der Kooy and Nagy, 1986). This method was successfully utilized to demonstrate axonal transport of ketanserin binding sites (Snowhill and Wamsley, 1983) in the medial forebrain bundle (MFB). Although these sites were originally identified as $5HT_2$ receptors (receptor sites specific for ketanserin binding), ketanserin has more recently been shown to bind to additional sites associated with monoamine (dopamine) transport (Leysen et al., 1987; Darchen et al., 1988). Transported ketanserin binding sites in the MFB must represent some labeling of the latter sites since destruction of the dopaminergic neurons in this pathway (with the neurotoxin 6-hydroxydopamine) greatly reduces the presence of ketanserin binding sites in the caudate-putamen (Wamsley and Dawson, unpublished observations).

Opiate and dopamine receptors also accumulate on both sides of the transected nigrostriatal and striatonigral pathways (Van der Kooy and Nagy, 1986). A presynaptic population (Filloux et al., 1988) of dopamine type 1 ($D_1$) receptors has been established to exist in the SNR. Lesion of cell bodies in the caudate-putamen (CP) with ibotenic acid eliminates $D_1$ receptor binding within the immediate boundaries of the lesion. This same procedure wipes out much of the $D_1$ receptor binding in the SNR. Direct administration of ibotenic acid to destroy cell bodies in the SNR, however, has virtually no effect on the $D_1$ receptor population in that structure. Thus, $D_1$ receptors are postsynaptic in the CP and presynaptic on striatonigral fiber terminals (presumably GABAergic) in the SNR.

Physiological evidence exists for the presence of presynaptic $D_2$ autoreceptors on dopaminergic projections in the nigrostriatal pathway (Bunney and Aghajanian, 1973; Nissbrandt et al., 1985). The presence of $D_2$ autoreceptors on the cell bodies in the SNC has been verified (Filloux et al., 1988). Lesion of cell bodies in this region, with ibotenic acid, eliminates $D_2$ receptor binding. This situation can be duplicated by lesion with 6-OHDA using conditions that make this neurotoxin specific for neurons containing dopamine. Identification of $D_2$ receptors on the terminals of these dopaminergic fibers in the CP has been more difficult to establish although some evidence exists (Van der Kooy and Nagy, 1986). An upregulation of postsynaptic $D_2$ binding (dennervation supersensitivity) may overwhelm the slight reduction in $D_2$ receptor binding in the CP following 6-OHDA lesion of the SNC.

Injection of 6-OHDA into the MFB has been used to supply evidence for the existence of transported β-adrenergic receptors in this pathway (Levin, 1982). These were identified as autoreceptors transported in catecholaminergic neurons and the receptors appear to be of the $\beta_1$ subtype.

An electrothermal lesion was used to indicate the possible transport of muscarinic cholinergic receptors in the fimbria–fornix pathway of the hippocampal formation and in the corpus callosum (Wamsley, 1983). Muscarinic sites accumulated on both sides of coagulated tissue in these two pathways, and these receptors reflected a pharmacology in keeping with what would be expected of muscarinic receptors. Lesion of incoming fibers into the hippocampus causes a slight, but significant increase in muscarinic cholinergic receptor binding in the hippocampus (Dawson et al., 1989; Dawson and Wamsley, 1990). Apparently, an upregulation of postsynaptic sites obscures any reduction in presynaptic receptors that occurs as a direct result of the lesion.

Serotonin uptake (transporter) sites associated with binding of the antidepressant imipramine have been identified in the MFB (Dawson et al., 1985). Imipramine appears to be specific in vitro for limiting 5HT uptake. In vivo

it is rapidly metabolized to a demethylated form known as desipramine (desmethyl imipramine) that is specific for blocking the uptake of norepinephrine. Transport of imipramine "receptors" to the axonal terminal would be expected in serotonergic neurons. Electrothermal destruction of the origin of many 5HT neurons (the dorsal raphe complex) prior to transection of the MFB, completely eliminated the accumulation of imipramine binding sites proximal (caudal) to the transection, but not distal. After synthesis and anterograde transport is interrupted, retrograde transport may still take place for a short time even though the destination (cell body) is gone.

## Future Trends

Axonal transport of receptors appears to be a common and necessary phenomenon for providing receptors to terminals regardless of the ultimate purpose. Evidence for the existence of presynaptic receptors that are transported from the cell body to the terminals is convincing. Identification of the actual nerve cell population propagating the receptors is not always so clear-cut, but the demonstration of receptor transport for heteroreceptors appears most secure.

Understanding the life cycle of a receptor and its regulation could be exploited as a means of influencing the responsiveness of neurons to the drug and neurotransmitter milieu. This could be useful in the development of new medications that bypass a direct interaction with a specific receptor system by augmenting or reducing the efficacy of substances acting at other sites.

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