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What is the function of receptor and membrane endocytosis at the postsynaptic neuron?

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This paper explores the implications of certain new developments in cell biology upon neuroscience. Until recently it was thought that neurotransmitters and neuromodulators had only one function, which was to stimulate their specific receptors at the cell surface. From here on, all activity was supposed to be effected by postsynaptic cascades. The discovery that membrane components, particularly G-protein-linked receptors, are not static but are subject to a massive and complex process of continual endocytosis, processing in the endosome system and recycling back to the external membrane, raises the question of its functional significance. In addition, it has been found that many neuromodulators such as polypeptides have their main locus of action inside the postsynaptic neuron. This review covers the role of the endocytic mechanism on receptor desensitization and resensitization, synaptic reorganization and plasticity, synaptic scaling and the possible repair of oxidative damage. The possible involvement of this system in Alzheimer's disease is discussed.

Keywords: endocytosis; G-protein-linked receptors; membrane recycling; synaptic plasticity; synaptic scaling; PI-3 kinase

1. INTRODUCTION

The classical account of the mode of operation of receptors for neurotransmitters (NT) and neuromodulators (NM) in the nervous system has been a static one. In this account the NT or NM molecule binds to its receptor in the plasma membrane of the neuron. This triggers the opening of an ion channel or the initiation of a postsynaptic cascade in the postsynaptic cell. On leaving the receptor molecule the NT or NM molecule is inactivated by metabolism or transport and the receptor molecule waits in the membrane until the next NT or NM molecule arrives, when the process is repeated. It is recognized that receptor molecules are eventually replaced but it is thought that the time-scale for this is slow. Consequently, the focus of neuroscience research has been on the activity of the receptor and the postsynaptic cascade.

However, recent advances in cell biology have considerably altered this picture. It has been determined in the case of G-protein-linked (and several other) receptors that binding a molecule of the NT or NM results in phosphorylation of the receptor protein. (A list of some of the receptors known to be endocytosed is given in Appendix A.) This leads to a conformational change, which uncovers an amino-acid sequence that acts as a signal for internalization of the receptor (Clague 1998). The receptor is taken up by a pit, usually clathrin coated, sometimes not, which then deepens to form a vesicle (Mukherjee *et al*. 1997). The neck of this is pinched off and sealed by a specific protein—dynamin (Bottomley *et al*. 1999). This process is very extensive—some 2000–3000 vesicles per hour can bud off in this manner from the membrane of a cultured cell (Bottomley *et al*. 1999). Not only receptors but the entire membrane is also subject to the endocytosis process. A typical membrane protein with a half-life of 10–20 h will make at least ten round trips to the endosome per lifetime (Kelly 1999).

Upon endocytosis, the vesicle with its contents is trafficked to the early endosome and delivers its contents (receptor and in some cases ligand) into the very acidic interior (*ca*. pH 3.5). This acidity causes the ligand to separate from the receptor protein. From the early endosome, the receptor is moved to the late endosome where a triage process takes place. Some of the protein (possibly normal or repaired) is recycled back to the external membrane. The rest (possibly damaged) goes to the lysosome system where it is broken down (Moore *et al*. 1999). The fate of the ligand is known in the case of polypeptide NMs and hormones. These traffic to the nucleus to modulate transcription (Koenig & Edwardson 1997). As the latter say (p. 282) '. . . the purpose of endocytosis is to capture the ligand for subsequent use by the cell'. The fate of smaller ligands is unknown. There exist at present no data on this point. Koenig & Edwardson (1997) express doubts as to whether small molecules with low affinity could stick onto their receptors during endocytosis. However, the relationship between internalization and intrinsic activity is nonlinear (Szekeres *et al*. 1998).

The process of endocytosis can be relatively fast. Endocytosed β2 adrenergic receptors reach the endosome in 10 min (Cao *et al*. 1998) and dopamine D1 receptors in 4 min (Dumartin *et al*. 1998). Endocytosed nerve-growth-factor receptor molecules are completely recycled in 30 min (Zapf-Colby & Olefsky 1998). In the large synaptic terminals of goldfish retinal bipolar cells the entire external membrane is recycled every 2 min by an active endocytic cycle (Bretscher & Aguado-Velasco 1998*b*; Rouze & Schwartz 1998). Other molecules that play a role in endocytosis include arrestin (Carman & Benovic 1998; Iacovelli *et al*. 1999), rabaptin-5 and the growth-associated protein GAP-43 (Neve *et al*. 1998), and a special chaperone protein—receptor-associated protein (RAP) (Willnow 1998). A key role in endocytosis is played by phosphatidylinositol-3 kinase (Shpetner *et al*. 1996; Folli *et al*. 1997; Chibalin *et al*. 1998; Krugman &

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Welch 1998). This enzyme is also involved in neurite extension (Kimura *et al*. 1994).

2. THE FUNCTIONAL SIGNIFICANCE OF ENDOCYTOSIS OF RECEPTORS

G-protein-linked receptors are regulated by three processes (Carman & Benovic 1998):

(i) desensitization via G-protein-coupled kinases, which phosphorylate activated receptors and recruit arrestin to aid in receptor desensitization;
(ii) internalization (endocytosis), which is also facilitated by arrestin; the number of receptor molecules at the surface is a function of the relative weighting of endocytosis and recycling;
(iii) reduction in number; this may be absolute or secondary to redistribution to the interior of the cell.

However, endocytosis may have other functions.

(i) The phosphorylated desensitized receptor (and other proteins) cannot be dephosphorylated *in situ*. This has to be done in the endosome (Ferguson & Caron 1998). The resensitized dephosphorylated protein is recycled to the surface.

In some cases endocytosis is required for receptor desensitization only (e.g. secretin and conA receptors) and in other cases for resensitization only (e.g. adenosine A2 receptors) (Mundell & Kelly 1998). There are also instances where non-clathrin-coated vesicles are involved (e.g. angiotensin and muscarinic acetylcholine receptors) (Vickery & Von Zastrow 1999).

(ii) The process of membrane recycling can be directed to redistribute membrane components to areas of cellular growth, e.g. growing neurites or new synapses (Hu *et al*. 1993). KB cells respond to epidermal growth factor by producing 'prodigious' ruffles in the plasma membrane within minutes (Bretscher & Aguado-Velasco 1998*a*). The interior of the ruffle is formed by actin polymerized by Rac (a Ras-like GTPase). The new surface membrane comes from the endosome by directed recycling of the plasma membrane. Thus, the endosome membrane may act as a 'membrane bank' for the external membrane. This process may also be involved in the very rapid growth of dendritic spines (Engert & Bonhoeffer 1999), during which large quantities of membrane have to be supplied for the new spine, although there is as yet no direct evidence for this.
(iii) All receptor (and other membrane) proteins and lipids are subject to continual oxidative attack by reactive oxygen species produced by a number of enzymes in the cell. This damage needs to be repaired. It had previously been assumed that this was done by repair enzymes *in situ* in the membrane. However, it is possible that this repair may be effected in the endosome–lysosome system. The ubiquitin–proteosome mechanism is involved in this process (Levkowitz *et al*. 1999). Ubiquitin is a 76-amino-acid polypeptide that binds to proteins to be degraded. Its targets include oxidatively damaged proteins (Figueiredo-Pereira & Cohen 1999) and it is involved in degradation of membrane proteins (Kornitzer & Ciechanover 2000). The ubiquitin–protein complex is formed in the endosome system, where it is then recognized by the 26S-proteasome enzyme (a large multicatalytic protease), and is degraded in the lysosome system. Grune & Davies (1997) confirm that proteosomes are concerned with the removal of oxidized proteins, which plays an essential role in the cellular defence against oxidative attack.

3. ENDOCYTOSIS IN NEURONS

The soma and dendrites of neurons have extensive networks of tubular early endosomes. Axons have these only in terminals and in *boutons-en-passage*. Late endosomes predominate in the cell body. These structures are visibly highly mobile (Parton *et al*. 1992).

Dendritic spines contain coated vesicles and multivesicular bodies suggesting local endocytic activity (Spacek & Harris 1997; and see Harris 1999).

Turrigiano & Nelson (1998) have introduced the concept of 'synaptic scaling'. In this the change in rate and ratio between rates of insertion of (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors into and removal from the synaptic membrane by endocytosis and recycling results in modulation of synaptic strength at all or many of a neuron's synapses. There is direct evidence that AMPA receptors are cycled through an N-ethylmaleimide-sensitive fusion protein (NSF)-dependent pathway (Lledo *et al*. 1999; Nishimune *et al*. 1998; Noel *et al*. 1999; Lissin *et al*. 1999; Lüthi *et al*. 1999; Lüscher *et al*. 1999; Morales & Goda 1999). This needs concomitant activation of *N*-methyl-D-aspartate receptors (Carroll *et al*. 1999). In cortical neurons synaptic scaling is modulated (*inter alia*) by brain-derived neurotrophic factor. Also endocytosis (via PI-3 kinase) is involved in neurite elongation (Kimura *et al*. 1994). There is a negative feedback between membrane tension and the endocytosis rate that operates in cells (e.g. during mitosis) (Raucher & Sheetz 1999).

De Wit *et al*. (2000) have reported that hydrogen peroxide (a freely diffusible molecule produced by several neuronal enzymes including cyclo-oxygenase and nitric acid synthase in the glutamate postsynaptic site) inhibits endocytosis of the epidermal-growth-factor receptor in human fibroblasts at an early stage that involves mono-ubiquitation of the receptor protein. This provides further evidence that the control of endocytosis, and thus synaptic plasticity, may be mediated in part by redox mechanisms involving hydrogen peroxide as a retrograde messenger (Smythies 1997, 1999).

In Alzheimer's disease, in which the brain is under severe oxidative stress from the oxidant β-amyloid protein, there is a 2.5-fold increase in the total endosome volume in cortical neurons suggesting increased endocytic activity (Cataldo *et al*. 1996, 1997). Endosomes are the site of internalization of amyloid precursor protein (and probably of β-amyloid formation) and of apolipoprotein E, both relevant to Alzheimer's disease. Physiological levels of the toxic oxidant β-amyloid protein stimulate PI-3 kinase activity (Luo *et al*. 1996). This increased rate of endocytosis in Alzheimer's disease may be in response to

the β-amyloid protein load, or to the need to repair the oxidatively damaged proteins in the brain, or both.

Furthermore, the neurofibrillary tangles typical of the disease contain insoluble hyperphosphorylated τ protein (Mesulam 1999). This may be secondary to a primary disorder in the dephosphorylating mechanism of the early endosome (Smythies 2000).

4. IMPLICATIONS FOR PHARMACOLOGY

The endocytosis-recycling system for receptors clearly plays a key role in receptor function and in synaptic plasticity and so offers a new venue for the site of action of known, and as yet unknown, drugs. For example, chlorpromazine acts on endosomes by inducing a redistribution of a clathrin-coated pit component AP-2 (Subtil *et al*. 1994). It also reduces transferrin but not interleukin-2 endocytosis and powerfully inhibits actin polymerization, and alters cell shape and motility in lymphocytes (Milzani & Dalledonne 1999). The action of many central nervous system (CNS)-active drugs is currently attributed entirely to their effects on receptors. An additional mode of action may be through the mechanisms described in this review whereby these receptors are endocytosed, processed and recycled. Some preliminary work has been reported in this area. Sojakka *et al*. (1999) found that isoproterenol inhibits transport from early to late endosomes and causes fragmentation of the late endosome. Henkel *et al*. (1999) report that substrates for and inhibitors of the intracellular enzyme Kex2 can be delivered by endocytosis. They suggest that this method might be of therapeutic use in the treatment of virus infections that depend on these proteases.

APPENDIX A

Among the receptors relevant to CNS function that are known to be endocytosed are the following (this list is not exclusive):

muscarinic ACh (Bernard *et al*. 1998; Sorensen *et al*. 1998; Szekeres *et al*. 1998);
α- and β-adrenergic (Cao *et al*. 1998; Hirasawa *et al*. 1998; Laporte *et al*. 1999);
dopamine (Dumartin *et al*. 1998);
serotonin (Hu *et al*. 1993);
A3 adenosine (Ferguson & Palmer 1999);
opioid (Ignatova *et al*. 1999; McConalogue *et al*. 1999);
receptors for various polypeptides and proteins (including hormones, growth factors and cytokines) (Grimes *et al*. 1996; Koenig & Edwardson 1997; Jans & Hassan 1998; Petrou & Tashjian 1998);
insulin (Di Guglielmo *et al*. 1998);
epidermal growth factor (Vieira *et al*. 1996; Skarpen *et al*. 1998);
nerve growth factor and TrkA (Grimes *et al*. 1996);
transferrin (Spiro *et al*. 1996);
IL-2 (Subtil *et al*. 1994);
polymeric IgA2 (Gibson *et al*. 1998);
AMPA receptors (Morales & Goda 1999; Carroll *et al*. 1999);
metabotropic glutamate (Doherty *et al*. 1999);
rabies virus (Lewis & Lentz 1999);
Na^+/K^+-ATPase (Chibalin *et al*. 1998);
Na^+/H^+ exchanger (Kurashima *et al*. 1998).

Note added in proof. Whistler *et al*. (1999) have found that addictive opiates differ from equally effective opiates with low addiction potential in that the latter are endocytosed after they bind to the μ receptor whereas the former are not. The authors suggest that this finding indicates 'a fundamental revision of our understanding of the role of receptor endocytosis in the biology of opiate drug action and addiction' (Whistler *et al*. 1999, p. 745).

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