

Appendix A14.6

Neurotransmitters and Receptors – what are they?

Several criteria have been established for a neurotransmitter, including (i) it must be synthesized and released from neurons; (ii) it must be released from nerve terminals in a chemically or pharmacologically identifiable form; (iii) it interacts with postsynaptic receptors and brings about the same effects as are seen with stimulation of the presynaptic neuron; (iv) its interaction with the postsynaptic receptor must display a specific pharmacology; (v) its actions are terminated by active processes (Kandel et al., 2000; Nestler et al., 2001). However, our growing appreciation of the complexity of the central nervous system (CNS), and of the existence of numerous molecules which exert *neuromodulatory* and *neurohormonal* effects has blurred the classical definition of neurotransmitters somewhat, and even wellknown neurotransmitters do not meet all these criteria under certain situations (Cooper et al., 2001).

Most neuroactive compounds represent small polar molecules which are synthesized in the CNS via local machinery, or are able to permeate the blood brain barrier. To date, over 50 endogenous substances that appear to be capable of functioning as neurotransmitters have been found in the brain. There are many plausible explanations for why the brain would need so many transmitters and receptor subtypes to transmit messages. Perhaps the simplest explanation is that the sheer complexity of the CNS results in many afferent nerve terminals impinging onto a single neuron. This requires a neuron to be able to distinguish the multiple information conveying inputs. Although this can be accomplished in part by spatial segregation, it is accomplished in large part by chemical coding of the inputs—that is, different chemicals convey different information. Moreover, the evolution of multiple receptors for a single neurotransmitter means that the same chemical can convey different messages depending on the receptor subtypes it acts upon. Additionally, the firing pattern of neurons is also a means of conveying information; thus, the firing activities of neurons in the brain differ widely and a single neuron firing at different frequencies can even release different neuroactive compounds depending on the firing rate (e.g. the release of peptides often occurs at higher firing rates than that which is required to release monoamines). These multiple mechanisms to enhance the diversity of responses—chemical coding, spatial coding, frequency coding—are undoubtedly critical in endowing the CNS with its complex repertoire of physiological and behavioral responses (Kandel et al., 2000; Nestler et al., 2001). Finally, the existence of multiple neuroactive compounds also provides built-in safeguards to ensure that vital brain circuits are able to function at all times.

Receptors

An essential property of any living cell is its ability to recognize and respond to external stimuli. Cell surface receptors have two major functions: recognition of specific molecules (neurotransmitters, hormones, growth factors, and even sensory signals) and activation of “effectors”. Binding of the appropriate agonist (i.e. the neurotransmitter or hormone) externally to the receptor alters the conformation (shape) of the protein. Cell surface receptors use a variety of membrane transducing mechanisms to transform an agonist's message into cellular responses. In neuronal systems, the most typical responses ultimately (in some cases rapidly, in others more slowly) involve changes in transmembrane voltage and hence neuronal changes in excitability. Collectively, the processes are referred to as transmembrane signaling or signal transduction mechanisms.

Interestingly, although increasing numbers of potential neuroactive compounds and receptors continue to be identified, it has become clear that the translation of the extracellular signals (into a form that can be interpreted by the complex intracellular enzymatic machinery) is achieved through a relatively small number of cellular mechanisms. Generally speaking, these transmembrane signaling systems, and the receptors that utilize them, can be divided into four major groups:

- a) those which are relatively self-contained in structure and whose message takes the form of transmembrane ion fluxes
- b) those which are multicomponent in nature and generate intracellular second messengers
- c) those that contain intrinsic enzymatic activity (e.g. receptor tyrosine kinases and phosphatases)
- d) those which are cytoplasmic and translocate to the nucleus to directly regulate transcription (gene expression) after they are activated by lipophilic molecules (often hormones) that enter the cell.

a) Ionotropic Receptors

The first class of receptors contains in their molecular complex an intrinsic ion channel. Receptors of this class include those for a number of amino acids including glutamate (e.g. the NMDA receptor), GABA (gamma aminobutyric acid via the GABA_A receptor), as well as the

nicotinic acetylcholine receptor, and the 5-HT₃ receptor. Ion channels are integral membrane proteins directly responsible for the electrical activity of the nervous system by virtue of their regulation of the movement of ions across membranes. Receptors containing intrinsic ion channels have been called “ionotropic” and are generally composed of four or five subunits which open transiently when neurotransmitter binds, allowing ions to flow into (e.g. Na⁺, Ca²⁺, Cl⁻) or out of (e.g. K⁺) the neuron, thereby generating synaptic potential. Often, the ionotropic receptors can be composed of different compositions of the different subunits, thereby providing the system with considerable flexibility. For example, there is extensive research into the potential development of an anxiolytic which is devoid of sedative effects by targeting GABA_A receptor subunits present only in selected brain regions. In general, neurotransmission mediated by ionotropic receptors is very fast, with ion channels opening and closing within milliseconds, and regulates much of the tonic excitatory (e.g. glutamate mediated) and inhibitory (e.g. GABA mediated) activity in the CNS; many of the classical neurotransmitters (e.g. monoamines) exert their effects on a slower time scale and are therefore often considered to be modulatory in their effects.

b) G Protein Coupled Receptors

Most receptors in the CNS do not have intrinsic ionic conductance channels within their structure, but instead regulate cellular activity by the generation of various “second messengers”. Most receptors of this class do not directly interact with the various second messenger generating enzymes but transmit information to the appropriate “effector” by the activation of interposed coupling proteins. These are the G protein coupled receptor families. The G protein coupled receptors (GPCRs, which comprise more than 80% of all known receptors in the body, and number ~ 300) all span the plasma membrane 7 times. The amino terminus is on the outside of the cell and plays a critical role in recognition of the ligand; the carboxy terminus and 3rd intracellular loop are inside the cell, and regulate not only coupling to different G proteins, but also “cross-talk” between receptors, and desensitization.

G proteins are so named because of their ability to bind guanine nucleotides, guanosine triphosphate (GTP) and guanosine diphosphate (GDP). Receptors coupled to G proteins include those for catecholamines, serotonin, acetylcholine, various peptides, and even sensory signals such as light and odorants. Multiple subtypes of G proteins are known to exist, and they play critical roles in amplifying and integrating signals.

Autoreceptors and Heteroreceptors

Heteroreceptors are receptor subtypes which are present on cells that do not contain an endogenous ligand for that particular receptor subtype (e.g. a serotonergic receptor located on a dopaminergic neuron). By contrast, *autoreceptors* are receptors located on cells which contain the endogenous ligand for that particular receptor (e.g. a serotonergic receptor on a serotonergic neuron). There are two major classes of autoreceptors which play very important roles in fine tuning neuronal activity. Somatodendritic autoreceptors are present on cell bodies and dendrites, and play critical roles in regulating the firing rate of neurons. In general, activation of somatodendritic autoreceptors (e.g. α_2 adrenergic receptors for noradrenergic neurons, 5-HT_{1A} receptors for serotonergic neurons, or D₂ receptors for dopaminergic neurons) inhibits the firing rate of the neurons by opening K⁺ channels, and by reducing cAMP levels. On the other hand, nerve terminal autoreceptors play an important role in regulating the amount of neurotransmitter released per nerve impulse, generally by closing nerve terminal Ca²⁺ channels. Both of these types of autoreceptors are typically members of the G-protein coupled receptor family.

GPCR Regulation and Trafficking

The mechanism by which G protein-coupled receptors (GPCRs) translate extracellular signals into cellular changes was once envisioned as a simple linear model. It is now known, however, that the activity of GPCRs is subject to at least 3 additional principal modes of regulation: desensitization, down-regulation, and trafficking (Carman and Benovic, 1998). *Desensitization* refers to the process by which cells rapidly adapt to stimulation by agonists, and is generally believed to occur by two major mechanisms: homologous and heterologous. *Homologous desensitization* is receptor-specific; that is only the receptor actively being stimulated becomes desensitized. This form of desensitization occurs via a family of kinases known as G protein coupled kinases (GRKs). When a receptor activates a G protein and causes dissociation of the α subunit from the $\beta\gamma$ subunits, the $\beta\gamma$ subunits are able to provide an “anchoring surface” for the GRKs to allow them to come into proximity of the activated receptor and phosphorylate it. This phosphorylation then recruits another family of proteins known as arrestins, which physically interfere with the coupling of the phosphorylated receptor and the G protein, thereby dampening the signal. This form of desensitization is very rapid, and usually transient (i.e., the receptors get dephosphorylated and return to the baseline state). However, if the stimulation of the receptor is excessive and prolonged, it leads to an internalization of the receptor, and often its degradation, a process referred to as downregulation (*vide infra*). *Heterologous desensitization* is not receptor-specific and is mediated by second messenger kinases such as PKA and PKC. Thus, when a receptor activates PKA, the activated PKA is

capable not only of phosphorylating that particular receptor (and thereby desensitizing it), but also other receptors which are present in proximity and have the correct phosphorylation motif, thereby producing heterologous desensitization.

Upon prolonged or repeated activation of receptors by agonist ligands, the process of receptor down-regulation is observed. *Down-regulation* is associated with a reduced number of receptors detected in cells or tissues, thereby leading to attenuation of cellular responses (Carman and Benovic, 1998). The process of GPCR sequestration is mediated by a well characterized endocytic pathway involving the concentration of receptors in clathrin-coated pits and subsequent recycling back to the plasma membrane (Tsao and von Zastrow, 2000). Endocytosis can thus clearly serve as a primary mechanism to attenuate signaling by rapidly and reversibly removing receptors from the cell surface. However, emerging evidence suggests additional functions of endocytosis and receptor trafficking in mediating GPCR signalling via certain effector pathways, most notably MAP kinase cascades. There is also evidence that endocytosis of GPCRs may be required for certain signal transduction pathways leading to the nucleus (Tsao and von Zastrow, 2000). These diverse functions of GPCR endocytosis and trafficking are leading to unexpected insights into the biochemical and functional properties of endocytic vesicles. Indeed, there is considerable excitement about our growing understanding of the diverse molecular mechanisms for signaling specificity and receptor trafficking, and the possibility that this could lead to highly selective therapeutics.

c) Receptor Tyrosine Kinases

The receptor tyrosine kinases, as their name implies, contain intrinsic tyrosine kinase activity, and are generally utilized by growth factors and cytokines. Binding of an agonist initiates receptor dimerization, and transphosphorylation of tyrosine residues in its cytoplasmic domain (Patapoutian and Reichardt, 2001). The phosphotyrosine residues of the receptor function as binding sites for recruiting specific cytoplasmic signaling and scaffolding proteins. The ability of multiple effectors to interact with phospho-tyrosines is undoubtedly one of the keys to the pleiotropic effects that neurotrophins can exert. These pleiotropic and yet distinct effects of growth factors are mediated by varying degrees of activation of three major signaling pathways: the Ras/MAP kinase pathway, the PI-3 kinase pathway, and the phospholipase C- γ 1 pathways.

d) Nuclear Receptors

Nuclear receptors are transcription factors that regulate the expression of target genes in response to steroid hormones and other ligands. Many hormones (including cortisol, gonadal

steroids and thyroid hormones) are able to rapidly penetrate into the lipid bilayer membrane due to their lipophilic composition, and thereby directly interact with these cytoplasmic receptors inside the cell. Upon activation by a hormone, the nuclear receptor-ligand complex translocates to the nucleus, where it binds to specific DNA sequences referred to as hormone responsive elements (HREs), and subsequently regulate gene transcription (Mangelsdorf et al., 1995; Truss and Beato, 1993). Nuclear receptors often interact with a variety of coregulators that promote transcriptional activation when recruited (coactivators), and those that attenuate promoter activity (corepressors).

References

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