

## G-protein-coupled Receptors, Part 1; cyclic AMP

Chemical synaptic transmission is a special case of the general process by which cells in multicellular organisms (and sometimes even normally unicellular organisms like bacteria and amoebae) communicate with one another. In all these cases a similar suite of strategies is used: the “sending” (or signaling) cell releases molecules that cannot cross membranes and therefore bind to specific receptor proteins in the membrane of the “receiving” (or responding) cell; the signaling cell releases membrane-permeable substances that diffuse through the membrane of the responding cell and bind to specific *intracellular* receptor proteins; or the signaling cell has specific proteins in its plasma membrane that can bind directly to receptor proteins in the plasma membrane of the responding cell. These possibilities are summarized in Fig. 7.3 of Purves et al., *Neuroscience*, 3<sup>rd</sup> Ed. In each case, binding of a signaling molecule to a receptor sets in motion a chain of events in the responding cell that ultimately alters its components, function, and “behavior”.

The signaling cell can be adjacent to the responding cell (as in synaptic transmission); nearby, with the signaling molecule diffusing to the responding cell (called “paracrine signaling”); or some distance away, with the signaling molecule carried to the responding cell through the bloodstream (called “endocrine” signaling). (There’s an unusual form of signaling called “autocrine” signaling in which the signaling cell is also the responding cell; this happens in some neurons that have receptors for the transmitter molecule(s) that they release). Fig. 7.1 summarizes these mechanisms.

We’ve been discussing the ligand-gated ion channel type of receptors. There are other kinds of neurotransmitter receptors, including receptors that bind molecules that are membrane permeant, and receptors that are enzymes, which are activated by binding transmitter or, more

commonly, a signaling protein called a “cytokine” or “growth factor”. But perhaps the most important kind of receptor in the nervous system uses G-proteins and, usually, a second messenger system to indirectly alter ion permeabilities of the cell membrane, and often to change other properties of the target cell as well. (See Fig. 7.4). These and the enzyme-linked receptors are sometimes called "metabotropic receptors" because they alter the metabolism of the postsynaptic cell; they are also called G-protein-coupled receptors for reasons made clear below. Although the details of how this happens differ among different second messengers, of which there are known to be over a dozen, these systems all have a number of common features. In nearly every case, the complete functional system consists of 3 separate membrane associated proteins. One is the transmitter receptor *per se*; that is, it is the integral membrane protein that contains the neurotransmitter-binding site. Another is usually a membrane-associated enzyme that is activated by the receptor and that alters the concentration of an intracellular compound. And the third is a coupling protein that serves as a link between the receptor and the enzyme, or between the receptor and ion channels. These coupling proteins usually bind GTP or GDP--guanine nucleotides--and as a result they are called “G proteins”, which is short for “guanine-nucleotide-binding proteins”. The specificity in this system resides in the receptor protein, because the G proteins and enzymes are nonspecific, and can often be activated by more than one kind of receptor (and thus by more than one kind of transmitter). Because the physiological effect of a transmitter depends ultimately on the second messenger, two different transmitters can change the concentration of the same transmitter and thereby have similar effects on the “receiving” neuron.

The major advantage of these so-called “second messenger” systems is that they permit an enormous amplification of a signal inside a cell. As shown schematically in Fig. 7.2, the steps following activation of a typical G-protein coupled receptor result in several rounds of

amplification so that the final effect on the target cell is enormously greater than could be achieved by simply activating a single receptor molecule.

The oldest, most studied (but probably not most common) second messenger is cyclic AMP, a modified form of the nucleotide AMP. A number of transmitters, such as norepinephrine and dopamine, often induce an increased concentration of cAMP in their target neurons. The current model of how this happens, using NE as an example, is this (see Fig. 7.5 and 7.6 in Purves et al.). First noradrenaline binds to its receptor protein (which is called the  $\beta$ -adrenergic receptor). As a result of the binding, the receptor can interact with the quiescent form of the G protein, by diffusing in the plane of the lipid bilayer. This G protein consists of three subunits--called  $\alpha$ ,  $\beta$ ,  $\gamma$ --that bind GDP in their inactive state. When this inactive G protein trimer interacts with the activated beta-adrenergic receptor, the interaction catalyzes an exchange reaction in which GDP is replaced by GTP. This exchange in turn promotes the dissociation of the G protein trimer into the  $\alpha$  subunit and a  $\beta,\gamma$  dimer. The  $\alpha$  subunit, to which the GTP is bound, can now diffuse in the plane of the membrane until it contacts inactive adenylyl cyclase. This interaction, between the adenylyl cyclase and the  $\alpha$  subunit of the G protein, activates the adenylyl cyclase, so that it can now convert ATP to cAMP at the rate of several thousand reactions per second. This process continues until the GTP is hydrolyzed by the  $\alpha$  subunit back to GDP; the  $\alpha$  subunit is a slow GTPase, and so it essentially turns itself off after a time. This breakdown of GTP to GDP causes dissociation of the  $\alpha$  subunit and the adenylyl cyclase, which turns off the adenylyl cyclase and allows the other two subunits to rebind it to form the original trimer.

As we'll see later, this basic scheme--receptor activates G protein activates enzyme--is very common, although both the specific G protein and the specific enzyme can differ widely among neurons. A variant on the scheme happens in some cells. A different kind of receptor for

norepinephrine is called the  $\alpha$ -adrenergic receptor; binding of adrenaline to the alpha receptor actually lowers the cAMP levels in cells. Thus binding of adrenaline to the alpha receptor, or of dopamine to the D2 dopaminergic receptor (Fig. 7.6), has the opposite effect of binding to the beta receptor or of any signalling molecule that elevates cAMP. This *antagonism* is mediated by a different kind of G protein, called  $G_i$  for inhibitory G protein. (What do you think the G protein that's associated with the  $\beta$ -adrenergic receptor is called?  $G_s$  for "stimulatory G protein").  $G_i$  is also a trimer of 3 subunits, and the beta and gamma subunits are the same as in  $G_s$ . The  $\alpha$  subunit is different from the alpha subunit of  $G_s$  however, though both  $\alpha_i$  and  $\alpha_s$  bind GTP/GDP. The difference is that when  $G_i$  is activated by the receptor, and the alpha subunit dissociates, just as does the alpha subunit of  $G_s$ , this alpha subunit cannot bind to and activate the adenylyl cyclase. In fact it appears that both  $\alpha_i$  and the  $\beta\gamma$  dimers can inhibit adenylyl cyclase, and thereby block synthesis of cAMP.

To summarize, in the cAMP system binding of a signalling molecule to its receptor in the plasma membrane activates the exchange of GTP for GDP in a G protein, the dissociation of G protein into subunits, and the interaction of the G protein subunits with adenylyl cyclase, which either activates it to produce cAMP or inhibits it from doing so. There are many neurotransmitters that have been shown to raise the level of cAMP in cells, including noradrenaline, dopamine, histamine, and serotonin, while both noradrenaline and dopamine can also have the opposite effect.

Well, once cAMP concentration in the cell increases in response to the external signal, is that all there is? Not quite. As I mentioned last time the cell will respond either changing the activity of individual enzyme molecules or by changing the rate of synthesis of enzymes. cAMP can do both. The cAMP works by binding to the inactive form of an enzyme, called protein kinase A (PKA). This inactive form is a tetramer consisting of two identical catalytic subunits and two

identical regulatory subunits. When the cAMP level in the cell rises, it can bind to the regulatory subunit of PKA, and this causes dissociation of the tetramer into its component monomers--two regulatory subunits with cAMP attached, and two active catalytic subunits. (I.e., the regulatory subunits inactivate the catalytic subunits when they are all associated).

Now a protein kinase is a special class of enzyme; its substrate is other proteins (hence the “protein” part of its name; a protein kinase is an enzyme that “kinases”, that is, phosphorylates, other proteins). Thus the reaction it catalyzes is the transfer of a phosphate group from ATP to a serine, threonine or tyrosine in a protein. Enzymes that transfer phosphate from ATP to some other molecule are called kinases. That accounts for the P and K. The A in PKA is short for cyclic AMP. That is, PKA is activated by cAMP to transfer phosphates from ATP to protein substrates (PKA is a serine/threonine kinase; it attaches phosphates to serine or threonine only).

These active PKA subunits now can bind to and phosphorylate a lot of different enzymes. One of the reasons that cAMP has different effects on different kinds of cells is that different cells contain different proteins that are substrates for PKA. This basic scheme works in lots of different cells, not just neurons, although the outcome on the cell’s metabolism is different depending on what kind of cell it is. Thus although a particular hormone activates the same internal mechanism, increased cAMP, the consequence is can be quite different in different kinds of cells--liver, heart muscle, neurons, etc. That’s because why? Because different proteins in the different cells are activated by PKA. In neurons, some of the proteins phosphorylated by PKA are ion channel proteins. If an ion channel is phosphorylated, its ion conductance changes and  $V_m$  changes as a result. Basically, that's because the addition of a phosphate group, which has two negative charges, to a hydroxyl group on an amino acid side chain alters the overall charge of the ion channel protein, and thus causes it to change its shape, opening up the channel portion.

After the pulse of neurotransmitter ends and the transmitter diffuses away or is taken back up into the nerve terminal, what happens? First the transmitter dissociates from its receptor. The receptor no longer activates GTP exchange on the G protein, so the G proteins get stuck in the GDP-binding form, preventing it from interacting with adenylyl cyclase, and so adenylyl cyclase becomes inactive. Thus, cAMP is no longer being made. In fact cAMP is constantly degraded into the inactive molecule AMP by an enzyme called *phosphodiesterase*, so the cAMP levels begin to fall when cAMP is no longer being made. Once this happens, cAMP dissociates from the regulatory subunit of PKA, so that the PKA can reassemble--catalytic and regulatory subunits bind together. This of course inactivates the catalytic subunit so that it no longer can phosphorylate proteins. Finally there is a group of proteins that cut phosphate groups off the serine and threonine residues that PKA phosphorylated. These phosphatases the ion channels back to their original unphosphorylated forms which shifts the ion channel back into the non-conducting state). Thus, using this elaborate cascade of events, cells can rapidly respond to, and amplify, signals from the neurotransmitters that impinge upon their surface, and they can rapidly reverse the response when the signal is no longer present. Nevertheless, both the activation and inactivation process are slower than simply opening and closing an ion channel; thus transmitters that activate G-protein coupled receptors tend to have slower-to-start and longer-lasting effects on postsynaptic cells than transmitters that activate ligand-gated ion channels.

Finally, it's known that cAMP can have very long-lasting effects on cells by turning on the expression of certain genes. Not only does cAMP bind to and activate PKA, but PKA can bind to and phosphorylate (add a phosphate group to) another protein called CREB (which stands for Cyclic-AMP Response Element-Binding protein). CREB, when phosphorylated, can enter the nucleus and bind to DNA at places that contain a specific sequence of nucleotides (called the cyclic-AMP response element). This binding in turn activates transcription of the nearby genes,

production of mRNA, and then synthesis of new proteins. In nerve cells, the new proteins produced in response to increases in cAMP usually include ion channels. So cAMP can not only modulate the activity of ion channels in the short run, and thus alter the permeability of the cell membrane, it can actually change the protein composition of the membrane in the long run, which of course can greatly change the properties of the nerve cell, and thus increase or decrease the probability that it will produce action potentials when neurotransmitters bind to receptors on its surface. (Fig. 7.11 gives a brief overview of this process).

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