

Ligand-Gated Ion-Channels (Ionotropic Receptors)

Now let's consider the specifics of what happens when transmitters bind to their specific receptor proteins on the postsynaptic cell's plasma membrane. Studying this process is one of the hottest areas of neurobiology, and it combines techniques from molecular biology, physiology, and cell biology. It turns out that transmitter communication from pre- to post-synaptic cells is a specialized case of the general phenomenon of cell-cell communication and signal transduction that turns out to be important in all multicellular organisms-in the endocrine system, in development, in wound healing, in the immune response to foreign objects, etc. So even though neurobiologists come at this question from the perspective of understanding neuronal function, it's turning out that some of the details turn out to be similar in many different types of cells, not only neurons.

What happens when the transmitter binds to the receptor protein? Basically, all the receptors that I know about respond to the presence of transmitter in one of two ways, as we've previously indicated. Either the receptor protein is also an ion channel (or is directly coupled to an ion channel) or the receptor is coupled to a member of another class of proteins that are on the interior surface of the plasma membrane, called G proteins. In the first case, binding of the transmitter to receptor changes whether the channel associated with it is in the open (conducting) or closed (non-conducting) conformation. These kinds of receptors are called chemically-gated (or ligand-gated) ion channels or "ionotropic receptors". Like the nicotinic ACh receptor in muscle, such receptors contain ion channels that are more or less specific for which ions they allow to pass; binding of the transmitter usually opens the channel, allowing ions to flow through it (thus increasing the conductance of the cells membrane for ions). In a few rare cases, binding of the transmitter actually decreases the permeability of the ion channel, and decreases membrane conductance. So you should imagine that binding of one (or more) molecules of transmitter to the receptor protein induces it to shift its 3D shape (or conformation) slightly, altering the structure of the ion channel in a way that opens or closes it. Once the transmitter unbinds and diffuses away (or is broken down), the receptor shifts back to its resting conformation.

The second type of receptor is not coupled directly to an ion channel, but is coupled to an G protein. Usually binding of transmitter to the receptor activates the G protein, by causing it to dissociate into two subproteins. These in turn either directly activate ion channels or else they activate some enzyme in the neuron. The activated enzyme in turn catalyzes some reaction inside the cell and alters the concentration of some product of the enzymatic reaction. This product is usually called a second messenger (because the transmitter is thought of as the "first messenger" from the presynaptic neuron). The increased concentration of second messenger activates other enzymes inside the cell, and some of these enzymes may covalently modify membrane proteins, including proteins that serve as ion channels. This chemical modification alters the shape, and thus the permeability, of these ion channels, causing an increase or decrease in ion conductance of the cell membrane. Thus in ligand-gated channel receptors, the binding of transmitter to the receptor directly alters the conductance of the membrane to an ion, while in the G-protein-coupled receptors there are a number of intervening metabolic reactions between the binding of transmitter and the change in ion conductance; thus, the ion permeability is indirectly altered by the action of the transmitter. G-protein-coupled receptors are sometimes called "metabotropic receptors" to indicate that they induce metabolic changes in the postsynaptic cells. As you might guess, directly gated channels act faster than indirectly gated channels. In frog sympathetic ganglion, the fast epsp is caused by an ACh-gated ion channel, while the slow epsp and ipsp require second messenger activation by ACh-receptor activated enzyme systems; the late slow epsp induced by LHRH is also mediated by a second messenger system.

First, I want to discuss the directly gated ion channels then turn to the more complex and more diverse G-protein coupled receptor systems. The best known and most studied neurotransmitter is the nicotinic ACh receptor of skeletal muscle and related tissues; a close second is the nicotinic ACh receptor of neurons. ACh receptor was studied first by classical biochemistry because of two fortuitous tricks of nature. First, the electric organ of the electric marine ray *Torpedo* or the electric eel *Electrophorus* are immensely rich sources of AChR and second, the venom of the Formosan banded krait (*Bungarus multicinctus*-a sea snake) and related poisonous snakes such as the cobra (*Naja naja*) contain neurotoxic proteins that binds extraordinarily

specifically and extraordinarily tightly to the muscle AChR. Bungarotoxin, the protein from the banded krait, can be labelled radioactively and used as an assay for AChR. In addition, bungarotoxin or cobrotoxin can be used to affinity purify the AChR. After it was purified, it was shown that the AChR was a pentamer--i.e. a protein that has 5 different subunits--consisting of 4 different kinds of subunits in the stoichiometry $\alpha_2\beta\gamma\delta$ (Fig. 6.3). Each subunit is an integral membrane glycoprotein of MW 50-58K, as judged by cloning the genes and examining the predicted amino acid sequence. As this implies, AChR was the first receptor whose genes were cloned, in the 1980s, using predicted nucleotide sequences derived from the protein sequence. When the genes for the various subunits were cloned it was found that they had greater than 50% homology; i.e., they had very similar amino acid sequences. The usual conclusion that is drawn from this is that all the subunit genes are descended over evolutionary time from a common ancestral gene. Using muscle AChR genes as probes, people went looking for related genes and found a slew. In particular, the neuronal AChRs, as well as receptors for GABA (GABA_A), glycine, serotonin (some) and purines are all closely related to muscle AChRs (Fig. 6.4). This indicates that all are derived from one gene that existed a long time ago, that was duplicated many times, and then each duplicated copy could evolve independently to serve various functions. In each case, these ligand-gated ion channel receptors is a tetramer (4 subunits) or pentamer (5 subunits), and usually binds two molecules of neurotransmitter in order to open. Using a variety of techniques, it has been possible to conclude that all these kinds of receptors are integral membrane proteins and that each subunit seems to pass through the membrane 4 times (or sometimes 3 times with a fourth intramembrane loop—Fig. 6.4B)--with the N terminus extracellular and the C terminus intracellular. Thus the overall structure and function of the ionotropic receptors seems to be very similar, whether they are working at the neuromuscular junction or at synapses in the central nervous system. For this and other reasons, we have firm grounds for believing that the conclusions about synaptic function that are based on studies of the nmj are widely applicable to many other kinds of synapses throughout the nervous system.

It's possible to reconstruct the receptors in the frog (*Xenopus laevis*) oocyte, a large egg cell that is quiescent and easy to inject with cDNA or mRNA for the various subunits. It is then

possible to wait and assay the oocyte physiologically with a patch clamp to determine its ion channel properties. (Fortunately, normal oocytes don't have many ligand gated ion channels, themselves). When a Japanese group led by Numa injected the mRNA for various AChR subunits separately and in combination, they found that they only got a normal ACh gated ion channel if all 4 subunits (alpha, beta, gamma and delta) were present. That is, these subunits combine into both the ACh binding site and the ion selective channel--all 4 are both necessary and sufficient.

Subsequently, Numa and others have been carrying out site-directed mutagenesis of the AChR proteins. I.e., Just as we discussed how neurobiologists study the voltage-gated ion channels with a combination of molecular biology and patch clamping of *Xenopus* oocytes one alters the cDNA for the protein in order to change the amino acid sequence in some predictable way (e.g., changing a negatively charged amino acid into a neutral or a positively charged one). Then one injects the modified cDNA into the oocyte to see how the properties of the AChR are affected. In this way, they confirmed earlier work that each AChR has 2 ACh binding sites, one on each α subunit, and that negatively charged amino acids that lie on the extracellular region of the protein are responsible for ion selectivity, allowing cations (Na^+ , K^+ , and Ca^{2+}) to pass fairly non-specifically while excluding Cl^- and other anions.

While it's not correct to say that we completely understand how AChRs work, these muscle AChRs are by far the best understood of the ligand-gated channels, and the combination of molecular and patch clamp techniques allows them to be dissected functionally at the level of individual amino acids. Scientists are extending these kinds of studies to many other types of ligand gated receptors as well.

One of the surprising findings of the molecular biological search for receptor genes is the discovery that there are often many different genes that seem to have essentially identical functions. For instance, the neuronal nicotinic receptor usually contains only two kinds of subunits, called alpha and beta (although it may have 2 alphas and 3 betas, for a total of 5; i.e., it's pentameric just like the muscle AChR). You might think that it would be enough to have one gene for the alpha subunit and one for the beta subunit, but in fact at least 8 genes are known that encode alpha subunits and 4 that encode beta. Now the proteins these genes encode are very similar but slightly

different in their amino acid sequences, and it appears that any alpha type of subunit can pair up with any beta type. In some neurons, it's been shown that many different subunit types are expressed--e.g., alpha1, alpha4 and alpha 6. What this presumably means is that there are a large number of different possible combinations of alpha and beta subunits in one animal (at least $36=9 \times 4$) and even in one neuron. The function of this vast array of receptor subtypes is not understood. The presumption among workers in this field is that these different subtypes are subtly different in function. For instance, they may have different affinities for ACh and thus open at different concentrations or they have slightly different permeability properties for ions), so that the nervous system can "fine tune" the response of different neurons to ACh, but in fact, no one is really sure what's going on. Multiple receptor subtypes have been found for GABA, glycine, and glutamate receptors as well, so it's not unique to AChRs, and it's not an unusual phenomenon. (See Fig. 6.4 in Purves et al., *Neuroscience*). Interestingly, while the ACh receptors are permeable to cations, GABA and glycine receptors are permeable to Cl^- not to cations, and thus they are associated with inhibitory synapses, because E_{Cl} is almost always below the threshold for action potentials.

Although glutamate receptors don't seem to be in the same genetic "superfamily" as ionotropic ACh, GABA, and glycine receptors, they also appear to be pentamers of different subunits. There are least three different classes of glutamate receptors coupled to ligand-gated ion channels. These two are distinguishable by their affinity for particular chemicals. One, activated by N-methyl-D-aspartate, is called the NMDA-type of glutamate receptor. It is permeable to cations-- Na^+ , K^+ , and most importantly, Ca^{2+} . The other types are activated by alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) or by kainic acid (kainate), and these are called the AMPA/kainate receptors, respectively. They are permeable to Na^+ and K^+ , but not much to Ca^{2+} .

The following paragraph is an aside chock full of fascinating facts--Purves et al. discuss many of these topics in more detail. Chemical synapses are the Achilles heel of the nervous system because biochemical processes can be disrupted by chemicals. In particular, many medically important drugs and diseases affect synaptic transmission. We've already discussed some of these. ACh receptor blockers like curare (which is derived from plants) and snake venom toxins, paralyze

animals by preventing transmission at the nmj. Presumably the plant is protected from predators, while the snake paralyzes its prey, with these toxins (interestingly, snake toxins do block snake nmjs, so the toxin made in the venom gland must be sequestered from the snake's own nervous system.) Carnivorous marine snails of the genus *Conus* have a large number of neurotoxins in their venom, and they can paralyze and then eat fish much larger than themselves. A number of bacterial toxins--such as tetanus toxin or botulinum toxin--disrupt the release of synaptic vesicles, and thus cause paralysis and death. And many psychoactive drugs are either blockers (antagonists) or activators (agonists) of neurotransmitter receptors. Many of these affect ion channel-linked receptors including nicotine from tobacco (activates nicotinic ACh receptors); atropine (from the belladonna plant, also called deadly nightshade) activates muscarinic ACh receptors); tranquilizers such as Valium and Librium (benzodiazapines) activate GABA_A receptors and thus enhance inhibition, and so forth.

In addition several diseases interfere with synaptic transmission. One of the best studied is myasthenia gravis (severe muscle weakness), which turns out to be an autoimmune disease. That is, a person begins to make antibodies against his/her own muscle AChRs, which either blocks the binding of ACh or reduces the number of receptors. The basis of this disease was discovered in the 1970s when scientists who were trying to learn about the characteristics of ACh receptors, injected *Torpedo* AChRs into rabbits in order to induce the rabbits to make antibodies. The rabbits did make antibodies against the receptors but then developed a condition that looked much like myasthenia gravis in humans. When the blood from patients with MG was screened, it was found to contain anti-AChR antibodies also. The treatment for MG is thus twofold. Patients are given drugs that enhance synaptic transmission (such as blockers of acetylcholinesterase) and drugs that suppress the immune system and thus reduce the production of anti-AChR antibodies. As with many autoimmune diseases, patients with MG go through "relapsing and remitting" periods where the disease gets worse or better with no apparent reason.

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