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Signal transmission in ligand-gated receptors

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Summary

At synapses, a transmitter released from a pre-synaptic terminal binds to specific, ligand-gated receptors in the post-synaptic membrane to open up ion channels through the receptor molecules. The flow of ions through these channels generates electrical signals. Electrophysiological techniques have been used over the past 50 years to understand transmission of these signals at synapses. The most recent of these, the patch-clamp technique, allows very small picoamp currents through single-channel molecules to be recorded but gives little information about receptor structure or how drugs influence their function. Now, the subunits of most ligand-gated ion channels have been cloned and sequenced. Cryo-electronmicroscopy has revealed the structure of the ion channel activated by nicotinic agonists. It is pentameric and only a small part of it is in the membrane. In spite of this simple structure, the conductance of chloride channels activated by gamma-aminobutyric acid (GABAA channels) is very variable and can be increased markedly by drugs such as diazepam. Site-directed mutagenesis and labelling of cysteine residues in the open and the closed states are being used to define the residues that line the ion channel. Similar methods are being used to find the way in which drugs such as general anaesthetics modulate the function of GABAA receptors.

Key words: anaesthetics, conductance, diazepam, ethanol, GABA, ligand-gated ion channels.

Introduction

Rapid communication between neurons occurs at specialized areas of close contact called synapses. In response to an electrical signal there is a brief, rapid entry of calcium into the pre-synaptic terminal, a chemical neurotransmitter is sprayed into the synaptic cleft and the neurotransmitter binds to post-synaptic receptor proteins embedded in the target cell membrane. When the neurotransmitter binds to the receptor, it induces a conformational change in the receptor so that an ion channel opens and ions can flow across the post-synaptic membrane. It is the flow of ions that generates an electrical signal in the post-synaptic cell. Neurotransmitters can either excite or inhibit a target cell and hence are classified as either excitatory or inhibitory. But the specificity really resides in the ‘receptor’ rather than in the transmitter: acetylcholine can act as either an excitatory or an inhibitory transmitter, depending on the nature of the receptor to which it binds. Receptors that form ion channels following ligand binding are called ‘ionotropic receptors’ and the ion channels are called ‘ligand gated’.

More recently it has been discovered that neurotransmitters can activate another slower current in target cells that is mediated by a second messenger system that can indirectly alter the excitability of a cell. These receptors have been called ‘metabotropic’ to distinguish them from receptors that form ion channels directly. In this review, I will discuss only ionotropic receptors.

The introduction of intracellular recording techniques almost 50 years ago enabled the post-synaptic potentials generated by the movements of ions across an activated post-synaptic receptor to be recorded. Some of the first such events to be recorded were at the skeletal neuromuscular junction where acetylcholine is released from a motor nerve terminal and activates nicotinic acetylcholine receptors (nAChR) in the muscle membrane. In the early days it was shown that stimulation of the motor nerve caused a localized current to flow into the muscle cell. It was later shown that this was carried mainly by sodium ions. The movement of Na+ into the muscle makes the inside of the cell more positive (depolarized) and this activates voltage-sensitive Na+ channels to set off an action potential. Similar events occur at all excitatory synapses. The transmitter (e.g. acetylcholine or glutamate) triggers an inward movement of cations (Na+ or Ca2+) that depolarizes the target cell. At inhibitory synapses the neurotransmitter usually causes an inward movement of chloride ions. The inward movement of negative charge makes the inside of the cell more negative (hyperpolarized) and reduces the effect of an inward movement of cations caused by an excitatory neurotransmitter or an action potential.

Approximately 40 years ago, two electrode voltage clamp techniques were developed and this allowed definition of the ions that were flowing across activated channels. It was found that channels formed by nAChR were approximately equally permeable to Na+ and K+, less permeable to Ca2+ and essentially impermeable to anions. Then the tight seal patch-clamp technique, introduced approximately 15 years ago, allowed the current flowing through a single channel to be recorded. The current is
extremely small, commonly several picoamps. For the first time it was possible to see a single channel opening and closing. In general, the transitions between open and closed states are extremely rapid, channels stay open or closed for milliseconds or longer, and they have a ‘main’ conductance state (the ratio of the current that flows to the driving force on the ions).

Although great progress has been made in measuring functional characteristics of ligand-gated ion channels electrically, definition of the structure of the molecules responsible has proved to be much more intractable. The earliest work was done on the nAChR which was shown to contain several subunits. Hydropathy analysis of the subunits indicated that they had four transmembrane segments commonly called M1, M2, M3 and M4. Sequence homologies led to the proposal that there is a ‘superfamily’ of ligand-gated ion channels which includes the nAChR, the gamma-aminobutyric acid (GABA\(_A\)) receptor, the glycine receptor and the 5-hydroxytryptamine type 3 (5HT\(_3\)) receptor. Homology at the primary sequence level and conserved structural features have led to the proposal that members of this family of receptors form similar topological arrangements in the membrane and use similar conformations to form an integral ion channel. Site-directed mutagenesis studies, photoaffinity labelling with channel-blocking drugs and the use of cysteine-substitution mutations indicate that the second hydrophobic region, M2, forms at least part of the ion channel.

The classical work of Nigel Unwin has given us a glimpse of the three-dimensional (3-D) structure of the nAChR. Cryo-electronmicroscopy shows a barrel formed by five subunits. The extracellular part of the barrel is large and forms a ‘vestibule’ which presumably bears the ligand binding site (or sites). There is a single kinked transmembrane helix in each of the five subunits. It has been suggested that the helix is formed by M2 and that the highly conserved leucine residues, located at the point where the helix bends, form the channel gate. Opening of the channel is associated with the movement of this leucine, presumably opening up a pathway for the movement of ions.

In the present brief review I will discuss several aspects of signal transmission across ligand-gated ion channels using examples from work in our laboratory, in the sequence in which the experiments were done. The main emphasis will be on chloride channels activated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA\(_A\) channels).

The topics I will focus on are: (i) how do some clinically useful drugs modulate the GABA\(_A\) channel; (ii) what determines channel conductance; and (iii) what residues form the ion channel.

**Effects of general anaesthetics on ligand-gated ion channels**

Many years ago I made the chance observation that ethanol made end-plate potentials bigger and slowed their time course. This started my enduring interest in the effects of ethanol, and other drugs with similar effects. Later, when we had worked out how to voltage clamp the muscle membrane, we discovered that ethanol slowed the decay of the current activated by ACh, whereas a range of general anaesthetics made the current decay more rapidly. Slowing current decay makes potential changes larger, whereas speeding up the decay has the opposite effect. While these effects were interesting, they were obtained at the neuromuscular junction, hardly a site that could be claimed to be a primary site of action of general anaesthetics responsible for their clinical effects. So we switched to synapses in the brain. It was known that barbiturates increased post-synaptic inhibition mediated by GABA so we looked for a GABA\(_A\) synapse in the central nervous system (CNS) where the post-synaptic membrane potential could be clamped. We chose the synapse between basket cell dendrites and the soma of pyramidal neurons in the CA1 region of the rat hippocampus. Here, we found that a range of general anaesthetics, including barbiturates, slowed the decay of the synaptic current, an effect which would be expected to increase post-synaptic inhibition and perhaps cause unconsciousness. We also found that diazepam, a benzodiazepine, slowed the decay of the synaptic currents. I will discuss how mutation of a single residue in M2 can abolish the potentiation of GABA responses caused by pentobarbitone.

**Conductance of GABA\(_A\) channels**

It is generally assumed that single-channel currents recorded using patch-clamp techniques are flowing through a single channel formed by several subunits as described for the nAChR by Unwin. It is also generally thought that the conductance of a channel is fairly constant. In fact, some K\(^+\) channels are classified by their conductance. Most channels seem to shift occasionally to lower conductance states (called substates) but these are generally infrequent and are often ignored. While recording single-channel currents activated by GABA in cultured hippocampal neurons and in hippocampal slices, we have been struck by the variability in their conductance. Sometimes, a channel seems not to be able to make up its mind what conductance it wants to be, and an example of this Hamlet-type behaviour is shown in Fig. 1. The GABA\(_A\) channels we have studied also show outward rectification: they have a higher conductance at depolarized than at hyperpolarized potentials. When we analysed the subconductance states at hyperpolarized and depolarized potentials, we found that there were fewer subconductance states at hyperpolarized than at depolarized potentials. In fact, the outward rectification could be explained by a voltage-dependent change in the number of subconductance levels. We suggested that the channel was composed of a number of synchronized non-rectifying, conducting pores and that the number of pores activated changed with membrane potential.

In other experiments in which GABA\(_A\) channels were activated in cell-attached patches on neurons in hippocampal slices, channel conductance was sometimes seen to progressively increase following injection of GABA into the tip of a recording pipette. Conversely, injection of the competitive GABA antagonist caused a progressive reduc-
tion in the conductance of channels activated by GABA. We are now finding that the conductance of GABA_\textsubscript{A} channels in outside-out patches from cultured hippocampal neurons depends on GABA concentration and that the conductance can be reduced progressively as bicuculline concentration is increased.

Other drugs that are thought to act at allosteric sites can also affect channel conductance. For example, channel conductance can be increased up to seven-fold by diazepam, especially if the initial conductance is low.\textsuperscript{15} This effect can be countered by the specific benzodiazepine antagonist, flumazenil.

Taken together, these observations show that the conductance of GABA_\textsubscript{A} channels is anything but constant. Although not a unique explanation, our results are consistent with the idea that channels might be able to open cooperatively and that we are looking at the opening of a variable number of such coupled channels when we see a ‘single’ channel with variable conductance. This concept is illustrated in the diagram in Fig. 2. A more detailed consideration of this multipore hypothesis is available elsewhere.\textsuperscript{16}

In order to look more closely at the GABA_\textsubscript{A} ion channel, we have been expressing human \(\alpha_1\) and \(\beta_1\) subunits of the human GABA_\textsubscript{A} receptor using the baculovirus/Sf9 expression system. Neither subunit expressed alone forms detectable functional channels, but the two expressed together give chloride currents activated by GABA that are blocked by bicuculline, penicillin and picrotoxin and potentiated by pentobarbitone.\textsuperscript{17,18} We have introduced mutations into the highly conserved M2 region and in the M1 region in order to identify the residues involved in ion channel formation and drug effects. Following some mutations, Sf9 cells did not respond to GABA. This could have been because the channel was non-functional, because the GABA binding site was altered, or because functional receptors had not made it to the plasmalemma. In order to distinguish between these possibilities, we measure binding of labelled muscimol (a specific agonist of the GABA_\textsubscript{A} receptor). If the muscimol binds, we conclude that there are correctly assembled receptors in the plasmalemma with intact GABA binding site. We also detect the presence of the \(\alpha_1\) subunit using a fluorescently labelled antibody (bd24) to the \(\alpha\) subunit. I will describe here two mutations that have given interesting new information about the GABA_\textsubscript{A} receptor.

The conserved leucine in M2

All the receptors of the superfamily have a hydrophobic leucine residue near the middle of M2 at the \(\gamma^\prime\) position.\textsuperscript{19}
As mentioned in the previous section, this was the residue that Nigel Unwin suggested was at the kink in M2 and involved in channel gating. It had been shown that mutations of this residue in several members of the superfamily produce changes in the sensitivity of the receptor to agonists and antagonists and in the rate of desensitization. Replacing the 9 leucine residue with polar amino acids, serine or threonine, in homomeric α1 neuronal nACH receptors slowed the rate of decay of the whole-cell current activated by ACh, and increased the apparent agonist affinity by ~160-fold. Similar results were obtained in the homomeric 5HT3 receptor. In the heteromeric nACHR, desensitization was slowed when receptors contained two or more subunits carrying a 9 mutation. We mutated the 9 leucine in M2 to threonine in our α1β1 GABA_A receptors. When the mutation was in the α subunit alone, the response to GABA was reduced in amplitude, and desensitization was much slower than normal. No response to GABA was seen when the mutation was in the β subunit, whether in combination with mutated or normal α subunits. This was not due to lack of expression of subunits nor to the lack of GABA binding. However, the most striking effect of the mutation was the appearance of a large chloride current in cells not exposed to GABA. This current was apparently coming from constitutively open GABA_A receptors because it could be blocked by penicillin. Replacement of the hydrophobic leucine residue with the polar residue, threonine, had somehow provided a channel for chloride ions across the receptor. The result fits with Unwin’s hypothesis that the leucine acts as a gatekeeper of the channel but clearly does not prove it. It is possible that the mutation acts in some other way.

A mutation that abolishes potentiation by pentobarbitone

One of the mutations we tested influenced the effects of pentobarbitone. As mentioned in the previous section, the current generated by GABA in Sf9 cells expressing α1 and β1 subunits was potentiated by pentobarbitone. When the threonine residue at the 12 position in M2 was mutated to glutamine in the α subunit but not the β subunit, the current generated by GABA was still potentiated by pentobarbitone. However, the same mutation in the β subunit abolished the potentiating action of pentobarbitone, whether or not the α subunit carried the mutation. This observation suggests that, in spite of the high degree of sequence homology, especially in the M2 region, the two subunits are not functionally similar.

Conclusions

During the past 50 years, great progress has been made in understanding the function of ligand-gated ion channels. New advances are being made in understanding electrical events at synapses in terms of conformational changes in receptors. If the binding sites can be located for useful and widely used drugs such as general anaesthetics and benzodiazepines that were discovered serendipitously, it may be possible to design more selective drugs with similar action but free from unwanted side effects. The next 10 years holds great promise.

References


