4 Receptors: structure and function

In this chapter we discuss the structure and function of receptors. Drug action at receptors is discussed in Chapter 8 and in other chapters throughout the text.

4.1 Role of the receptor

Receptors are proteins which are, by far, the most important drug targets in medicine. They are implicated in ailments such as pain, depression, Parkinson's disease, psychosis, heart failure, asthma, and many other problems. What are these receptors and what do they do?

In a complex organism there has to be a communication system between cells. After all, it would be pointless if individual heart cells were to contract at different times. The heart would then be a wobbly jelly and totally useless in its function as a pump. Communication is essential to ensure that all heart muscle cells contract at the same time. The same is true for all the organs and tissues of the body if they are to operate in a coordinated and controlled fashion.

Control and communication come primarily from the brain and spinal column (the **central nervous system**), which receives and sends messages via a vast network of nerves (Fig. 4.1). The detailed mechanism by which nerves transmit messages along their length need not concern us here (see Appendix 4). It is sufficient for our purposes to think of the message as being an electrical pulse which travels down the nerve cell (**neuron**) towards the target, whether that be a muscle cell or another neuron. If that was all there was to it, it would be difficult to imagine how drugs could affect this communication system. However, there is one important feature that is crucial to our understanding of drug action. Neurons do not connect directly to their target cells. They stop just short of the cell surface. The distance is minute, about 100 Å, but it is a space that the electrical 'pulse' is unable to jump.

Therefore, there has to be a method of carrying the message across the gap between the nerve ending and the target cell. The problem is solved by the release of a chemical messenger called a **neurotransmitter** from the nerve cell (Fig. 4.2). Once released, this chemical messenger diffuses across the gap to the target cell, where it binds and interacts with a specific protein (receptor) embedded in the cell membrane. This process of binding leads to a series or cascade of secondary effects, which results either in a flow of ions across the cell membrane or in the switching on (or off) of enzymes inside the target cell. A biological response then results, such as the contraction of a muscle cell or the activation of fatty acid metabolism in a fat cell.

The first person to propose the existence of receptors was Langley in 1905. Up until that point, it was thought that drugs acted to prevent the release of the neurotransmitter from the neuron, but Langley was able to show that certain target cells responded to the drug nicotine, even when the neurons supplying those cells were dead.

So far, we have talked about cellular communication involving neurons and neurotransmitters, but cells also receive chemical messages from circulating **hormones**. Once again, receptors are responsible for binding these messengers and triggering a series of secondary effects.

We shall consider these secondary effects and how they result in a biological action in Chapter 5, but, for the moment, the important thing to note is that the communication system depends crucially on a chemical messenger. As a chemical process is involved, it should be possible for other chemicals (drugs) to interfere or interact with the process.

4.2 Neurotransmitters and hormones

There are a large variety of messengers that interact with receptors and they vary significantly in structure and complexity. Some neurotransmitters are simple molecules, such as monoamines (e.g. **acetylcholine**, **noradrenaline**, **dopamine**, and **serotonin)** or amino acids (e.g.

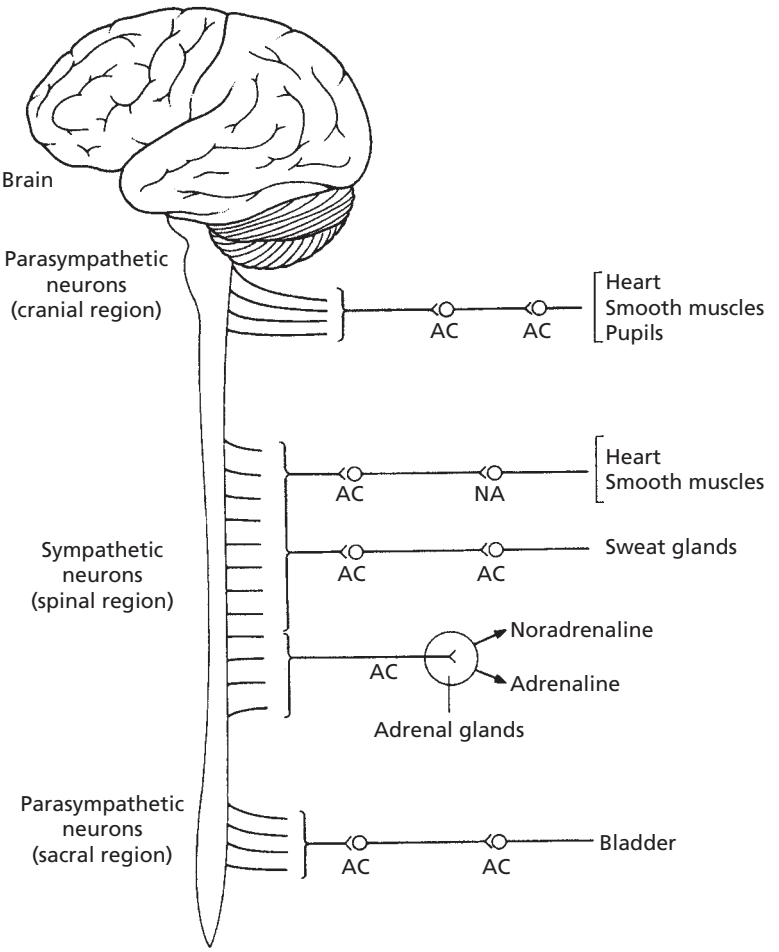


FIGURE 4.1 The central nervous system (AC = acetylcholine; NA = noradrenaline). Taken from Mann, J. (1992) *Murder, Magic, and Medicine*. Oxford University Press, with permission.

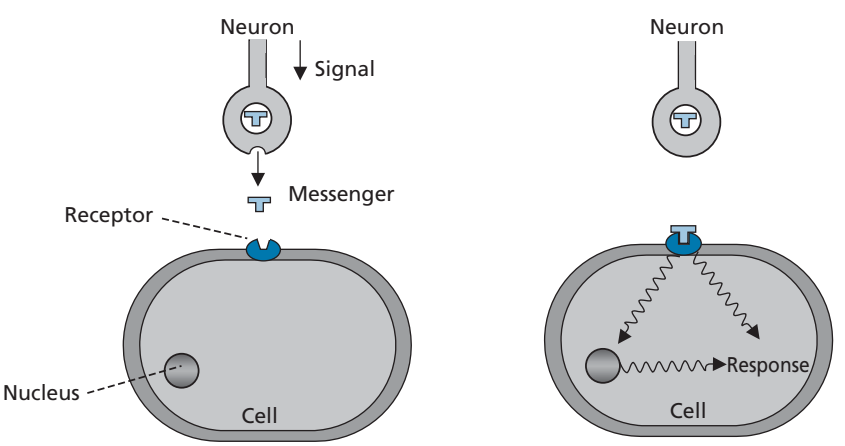


FIGURE 4.2 Neurotransmitters act as chemical messengers that bind to receptors and trigger reactions within a cell.

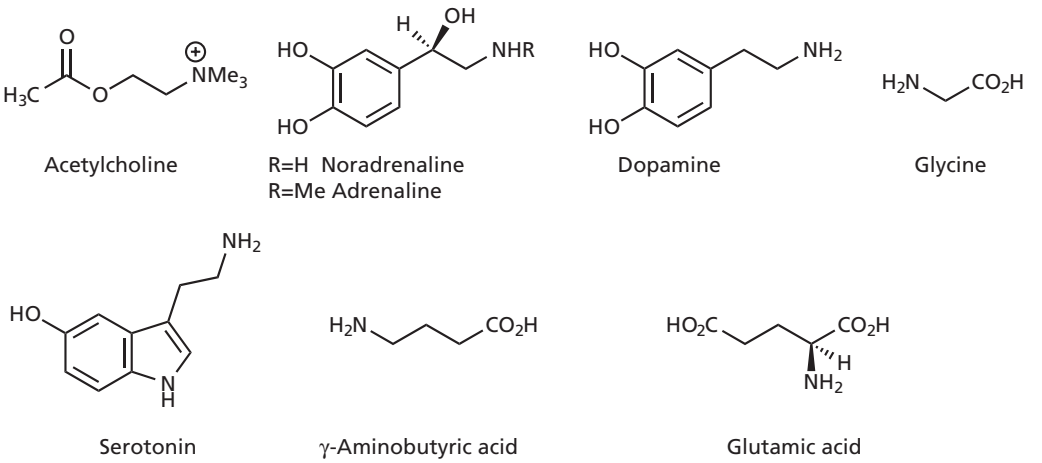


FIGURE 4.3 Examples of neurotransmitters and the hormone adrenaline.

γ-aminobutyric acid [GABA], **glutamic acid,** and **glycine**) (Fig. 4.3). Even the calcium ion can act as a chemical messenger. Other chemical messengers are more complex in structure and include lipids, such as **prostaglandins**; purines, such as **adenosine** or **ATP** (Chapter 6); **neuropeptides**, such as **endorphins** and **enkephalins** (section 24.8); peptide hormones, such as **angiotensin** or **bradykinin**; and even enzymes, such as **thrombin**.

In general, a neuron releases mainly one type of neurotransmitter, and the receptor which awaits it on the target cell will be specific for that messenger. However, that does not mean that the target cell has only one type of receptor protein. Each target cell has a large number of neurons communicating with it and they do not all use the same neurotransmitter (Fig. 4.4). Therefore, the target cell will have other types of receptors specific for those other neurotransmitters. It may also have receptors waiting to receive messages from chemical messengers that have longer distances to travel. These are the hormones released into the circulatory system by various glands in the body. The best known example of a hormone is **adrenaline**. When danger or exercise is anticipated, the adrenal medulla gland releases adrenaline into the bloodstream where it is carried round the body, preparing it for vigorous exercise.

Hormones and neurotransmitters can be distinguished by the route they travel and by the way they are released, but their action when they reach the target cell is the same. They both interact with a receptor and a message is received. The cell responds to that message and adjusts its internal chemistry accordingly, and a biological response results.

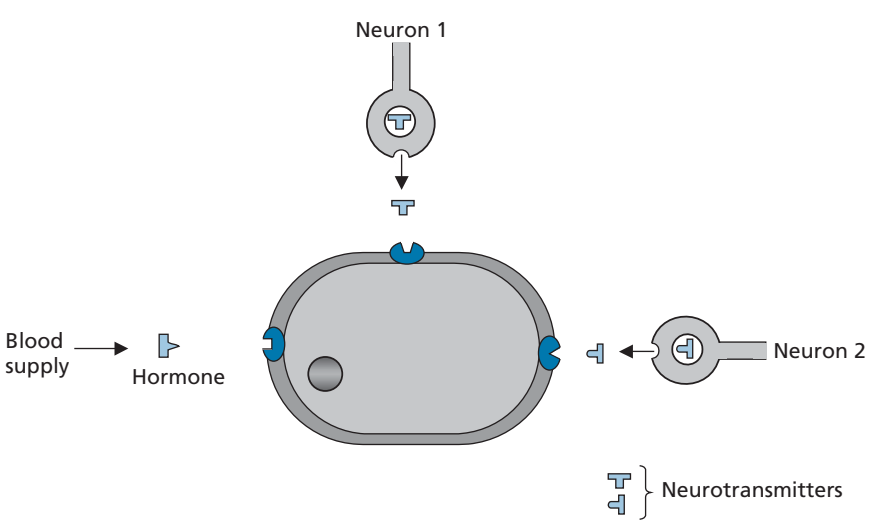


FIGURE 4.4 Target cell containing various receptors specific to different types of messenger.

4.3 Receptor types and subtypes

Receptors are identified by the specific neurotransmitter or hormone which activates them. Thus, the receptor activated by **dopamine** is called the **dopaminergic receptor**, the receptor activated by **acetylcholine** is called the **cholinergic receptor**, and the receptor activated by **adrenaline** or **noradrenaline** is called the **adrenergic receptor** or **adrenoceptor**.

However, not all receptors activated by the same chemical messenger are exactly the same throughout the body. For example, the adrenergic receptors in the lungs are slightly different from the adrenergic receptors in the heart. These differences arise from slight variations in amino acid composition; if the variations are in the binding site, it allows medicinal chemists to design drugs which can distinguish between them. For example, adrenergic drugs can be designed to be 'lung' or 'heart' selective. In general, there are various *types* of a particular receptor and various *subtypes* of these, which are normally identified by numbers or letters. Having said that, some of the early receptors that were discovered were named after natural products which bound to them, for example the muscarinic and nicotinic types of cholinergic receptor (section 22.4).

Some examples of receptor types and subtypes are given in Fig. 4.16. The identification of many of these subtypes is relatively recent and the current emphasis in medicinal chemistry is to design drugs that are as selective as possible for receptor types and subtypes so that the drugs are tissue selective and have fewer side effects.

4.4 Receptor activation

A receptor is a protein molecule usually embedded within the cell membrane with part of its structure exposed on the outside of the cell. The protein surface is a complicated shape containing hollows, ravines, and ridges. Somewhere within this complicated geography there is an area that has the correct shape to accept the incoming messenger. This area is known as the **binding site** and is analogous to the active site of an enzyme (section 3.3). When the chemical messenger fits into this site it 'switches on' the receptor molecule and a message is received (Fig. 4.5). However, there is an important difference between enzymes and receptors in that the chemical messenger does not undergo a chemical reaction. It fits into the binding site of the receptor protein, passes on its message, and then leaves unchanged. If no reaction takes place, what *has* happened? How does the chemical messenger tell the receptor its message and how is this message conveyed to the cell? The first thing to note is that when the messenger fits the binding site of the protein receptor it causes the binding site to change shape. This is known as an **induced fit**. This, in turn, has wider ramifications as there is a knock-on effect which causes the overall protein to change shape. But how does an induced fit happen and what is the significance of the receptor changing shape?

4.5 How does the binding site change shape?

As we have seen, the binding site of a receptor changes shape when a chemical messenger fits into it. This is not a moulding process in which the binding site wraps itself around the messenger. Instead, the induced fit is brought about by the intermolecular binding interactions that can take place between the messenger and the binding site. This is exactly the same process that occurs when a substrate binds to the active site of an enzyme (section 3.5.1), but, in this situation, no catalysed reaction follows binding.

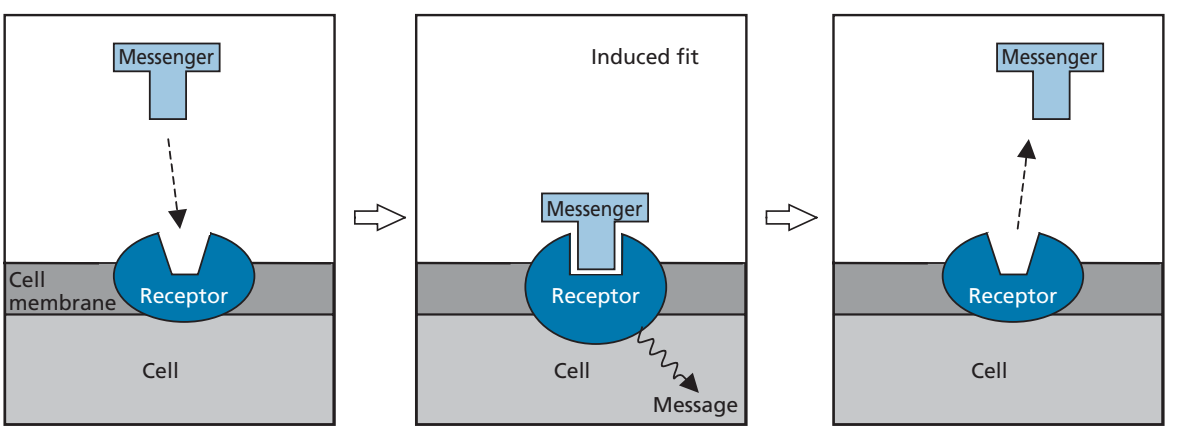


FIGURE 4.5 Binding of a chemical messenger to a protein receptor.

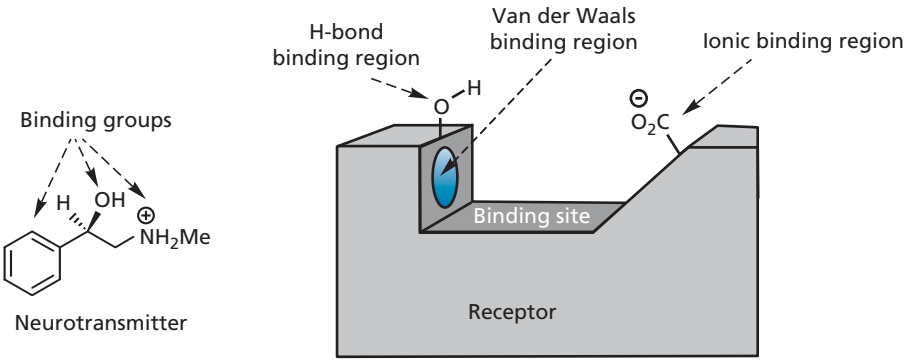


FIGURE 4.6 A hypothetical receptor and neurotransmitter.

To illustrate how binding interactions result in an induced fit, let us consider a hypothetical neurotransmitter and a hypothetical binding site as shown in Fig. 4.6. The neurotransmitter has an aromatic ring that can take part in van der Waals interactions, an alcohol OH group that can take part in hydrogen bonding interactions, and a charged nitrogen centre that can take part in ionic or electrostatic interactions. These functional groups are the messenger's **binding groups**.

The hypothetical binding site contains three **binding regions** which contain functional groups that are complimentary to the binding groups of the messenger. The messenger fits into the binding site such that intermolecular interactions take place between the messenger's binding groups and the receptor's binding regions (Fig. 4.7). However, the fit is not perfect. In the diagram, there are good van der Waals and hydrogen bond interactions, but the ionic interaction is not as strong as it could be. The ionic binding region is close enough to have a weak interaction with the messenger, but not close enough for the optimum interaction. The receptor protein therefore alters shape to bring the carboxylate group closer to the positively charged nitrogen and to obtain a stronger interaction. As a result, the shape of the binding site is altered and an induced fit has taken place.

The illustration shown here is a simplification of the induced fit process and, in reality, both the messenger and the binding site take up different conformations or shapes to maximize the bonding forces between them. As with enzyme–substrate binding, there is a fine balance involved in receptor–messenger binding. The bonding forces must be large enough to change the shape of the binding site, but not so strong that the messenger is unable to leave. Most neurotransmitters bind quickly to their receptors then 'shake themselves loose' once their message has been received.

We have now seen how a chemical messenger can cause an induced fit in the binding site of a receptor protein. However, this induced fit has a knock-on effect which alters the overall shape of the protein. It is this overall shape change that is crucial to the activation of a receptor and in its ability to trigger an amazing 'domino effect' which affects the cell's internal chemistry. This domino effect involves several different proteins and enzymes, and ultimately produces an observed biological effect. The process by which this takes place is called **signal transduction** and is covered in more detail in Chapter 5.

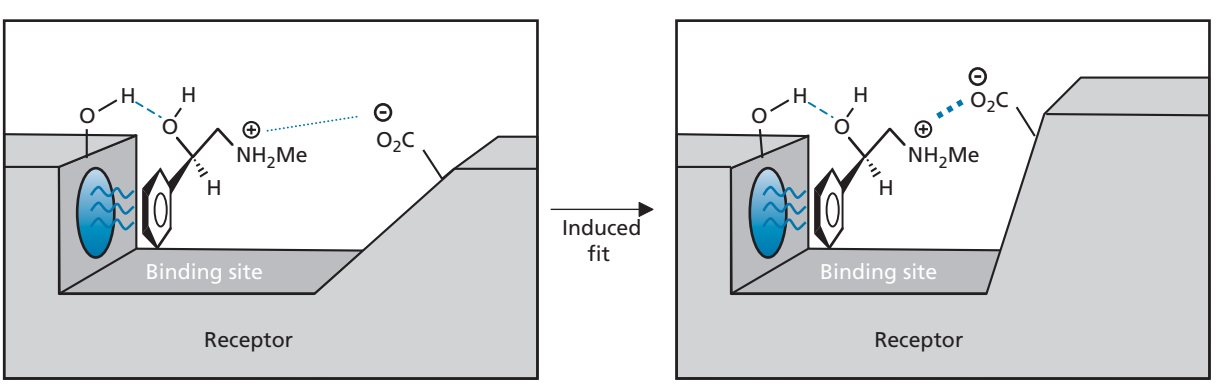


FIGURE 4.7 Binding of a hypothetical neurotransmitter to a binding site resulting in an induced fit.

Signal amplification is an important feature of this process as it means that a relatively small number of neurotransmitter molecules can have a dramatic effect on the cell's internal chemistry. In this chapter, we shall focus on the structure of different receptors and the process by which they are activated and trigger the signal transduction process.

There are three different types (or families) of membrane-bound receptors:

- ion channel receptors;
- G-protein-coupled receptors;
- kinase-linked receptors.

We shall consider each of these in turn in sections 4.6–4.8.

KEY POINTS

- Most receptors are membrane-bound proteins that contain an external binding site for hormones or neurotransmitters. Binding results in an induced fit that changes the receptor conformation. This triggers a series of events that ultimately results in a change in cellular chemistry.
- Neurotransmitters and hormones do not undergo a reaction when they bind to receptors. They depart the binding site unchanged once they have passed on their message.
- The interactions that bind a chemical messenger to the binding site must be strong enough to allow the chemical message to be received, but weak enough to allow the messenger to depart.
- Binding groups are the functional groups present on a messenger molecule which are used for binding it to the receptor binding site.
- Binding regions are regions of the receptor binding site which contain functional groups capable of forming intermolecular bonds to the binding groups of a messenger molecule.

4.6 Ion channel receptors

4.6.1 General principles

Some neurotransmitters operate by controlling ion channels. What are these ion channels and why are they necessary? Let us look again at the structure of the cell membrane.

As described in section 1.2.1, the membrane is made up of a bilayer of phospholipid molecules so the middle of the cell membrane is 'fatty' and hydrophobic. Such a barrier makes it difficult for polar molecules or ions to move in or out of the cell. Yet, it is important that these species should cross. For example, the movement of sodium and potassium ions across the membrane is crucial to the function of nerves (Appendix 4). It seems an intractable problem, but, once again, the ubiquitous proteins provide the answer by forming ion channels.

Ion channels are complexes made up of five protein subunits which traverse the cell membrane (Fig. 4.8). The centre of the complex is hollow and lined with polar amino acids to give a hydrophilic tunnel, or pore.

Ions can cross the fatty barrier of the cell membrane by moving through these hydrophilic channels or tunnels. But there has to be some control. In other words, there has to be a 'lock gate' that can be opened or closed as required. It makes sense that this lock gate should be controlled by a receptor protein sensitive to an external chemical messenger, and this is exactly what happens. In fact, the receptor protein is an integral part of the ion channel complex and is one or more of the constituent protein subunits. In the resting state, the ion channel is closed (i.e. the lock gate is shut). However, when a chemical messenger binds to the external binding site of the receptor protein, it causes an induced fit which causes the protein to change shape. This, in turn, causes the overall protein complex to change shape, opening up

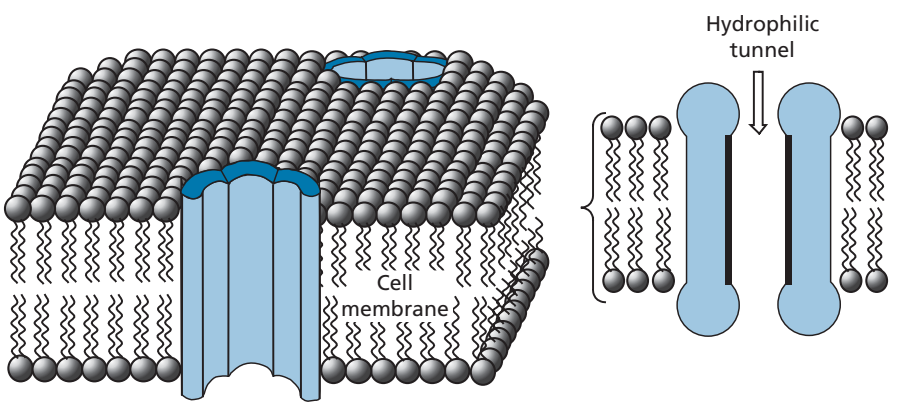


FIGURE 4.8 The structure of an ion channel. The bold lines show the hydrophilic sides of the channel.

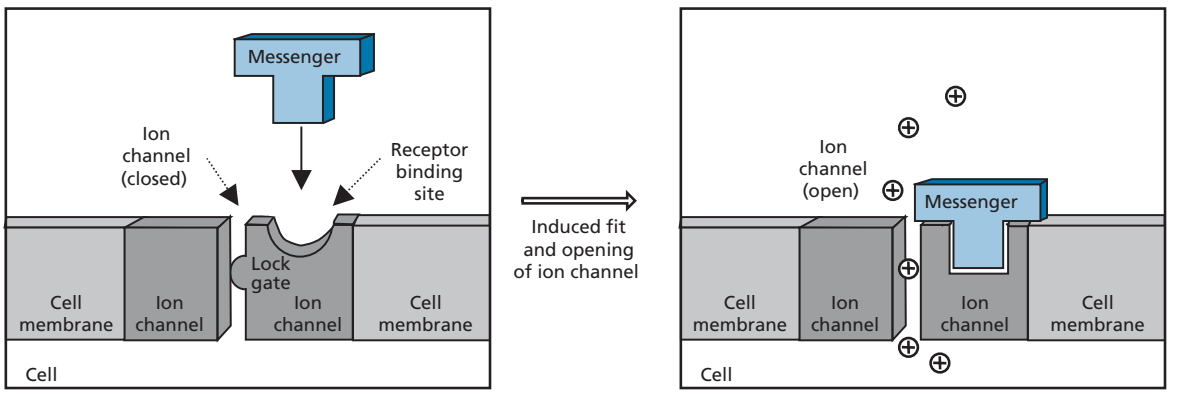


FIGURE 4.9 Lock-gate mechanism for opening ion channels.

the lock gate and allowing ions to pass through the ion channel (Fig. 4.9). We shall look at this in more detail in section 4.6.3.

The operation of an ion channel explains why the relatively small number of neurotransmitter molecules released by a neuron is able to have such a significant biological effect on the target cell. By opening a few ion channels, several thousand ions are mobilized for each neurotransmitter molecule involved. Moreover, the binding of a neurotransmitter to an ion channel results in a rapid response, measured in a matter of milliseconds. This is why the synaptic transmission of signals between neurons usually involves ion channels.

Ion channels are specific for certain ions. For example there are different cationic ion channels for sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) ions. There are also anionic ion channels for the chloride ion (Cl^-). The ion selectivity of different ion channels is dependent on the amino acids lining the ion channel. It is interesting to note that the mutation of just one amino acid in this area is sufficient to change a cationic-selective ion channel to one that is selective for anions.

4.6.2 Structure

The five protein subunits that make up an ion channel are actually **glycoproteins** (sections 2.5 and 10.7.1), but we will refer to them here as proteins. The protein subunits in an ion channel are not identical. For example, the ion channel controlled by the nicotinic cholinergic receptor is made up of five subunits of four different types [α (×2) β, γ, δ]; the ion channel controlled by the glycine receptor is made up of five subunits of two different types [α (×3), β (×2)] (Fig. 4.10).

The receptor protein in the ion channel controlled by glycine is the α-subunit. Three such subunits are present, all of which are capable of interacting with glycine. However, the situation is slightly more complex in the nicotinic ion channel controlled by the neurotransmitter acetylcholine. Most of the binding site is on the α-subunit, but there is some involvement from neighbouring subunits. In this case, the ion channel complex as a whole might be viewed as the receptor.

Let us now concentrate on the individual protein subunits. Although there are various types of these, they all fold up in a similar manner such that the protein chain traverses the cell membrane four times. This means that

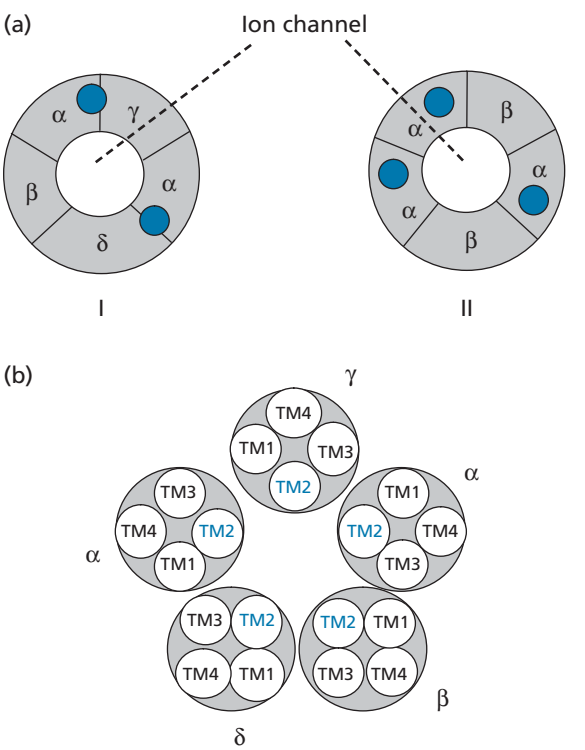


FIGURE 4.10 (**a**) Pentameric structure of ion channels (transverse view). I, ion channel controlled by a nicotinic cholinergic receptor; II, ion channel controlled by a glycine receptor. The coloured circles indicate ligand binding sites. (**b**) Transverse view of I, including transmembrane regions.

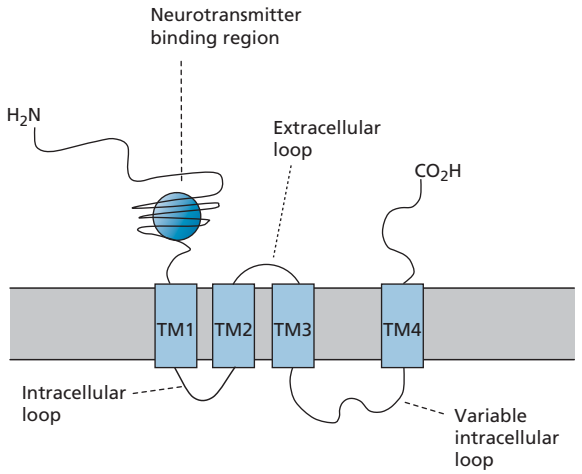


FIGURE 4.11 Structure of the four transmembrane (4-TM) receptor subunit.

each subunit has four transmembrane (TM) regions which are hydrophobic in nature. These are labelled TM1–TM4. There is also a lengthy *N*-terminal extracellular chain which (in the case of the α-subunit) contains the ligand-binding site (Fig. 4.11).

The subunits are arranged such that the second transmembrane region of each subunit faces the central pore of the ion channel (Fig. 4.10). We shall see the significance of this when we look at the next section.

4.6.3 Gating

When the receptor binds a ligand, it changes shape which has a knock-on effect on the protein complex, causing the ion channel to open—a process called **gating** (Fig. 4.12).

The binding of a neurotransmitter to its binding site causes a conformational change in the receptor, which eventually opens up the central pore and allows ions to flow. This conformational change is quite complex, involving several knock-on effects from the initial binding process. This must be so, as the binding site is quite far from the lock gate. Studies have shown that the lock gate is made up of five kinked α-helices where one helix (the 2-TM region) is contributed by each of the five protein subunits. In the closed state the kinks point towards each other. The conformational change induced by ligand binding causes each of these helices to rotate such that the kink points the other way, thus opening up the pore (Fig. 4.13).

4.6.4 Ligand-gated and voltage-gated ion channels

The ion channels that we have discussed so far are called **ligand-gated ion channels** as they are controlled by chemical messengers (**ligands**). There are other types of ion channel which are not controlled by ligands, but are instead sensitive to the potential difference that exists across a cell membrane—the **membrane potential**. These ion channels are present in the axons of excitable cells (i.e. neurons) and are called **voltage-gated ion channels.** They are crucial to the transmission of a signal along individual neurons and are important drug targets for local anaesthetics. A description of these ion channels is given in Appendix 4.

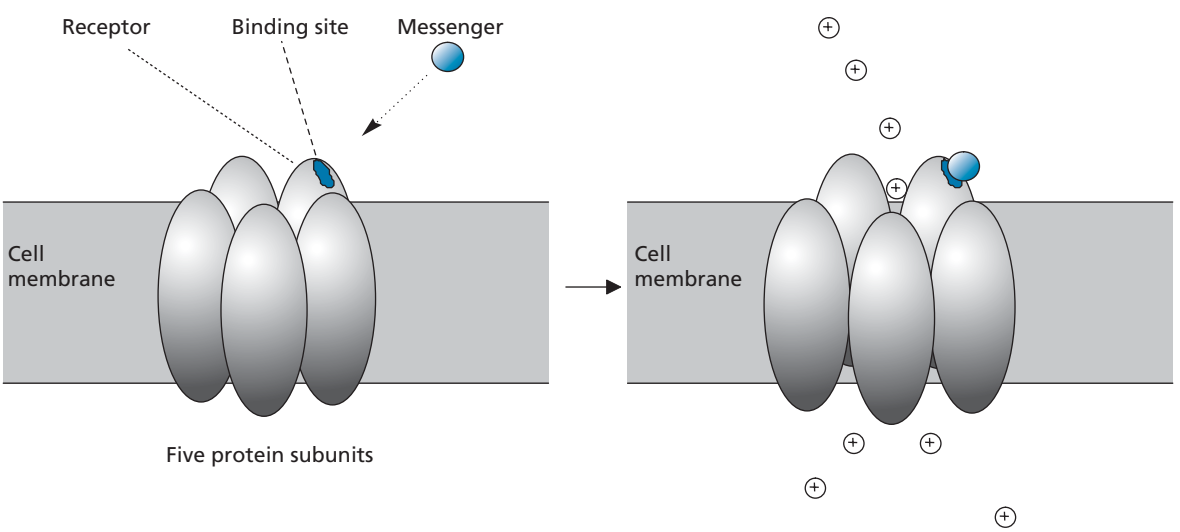


FIGURE 4.12 Opening of an ion channel (gating).

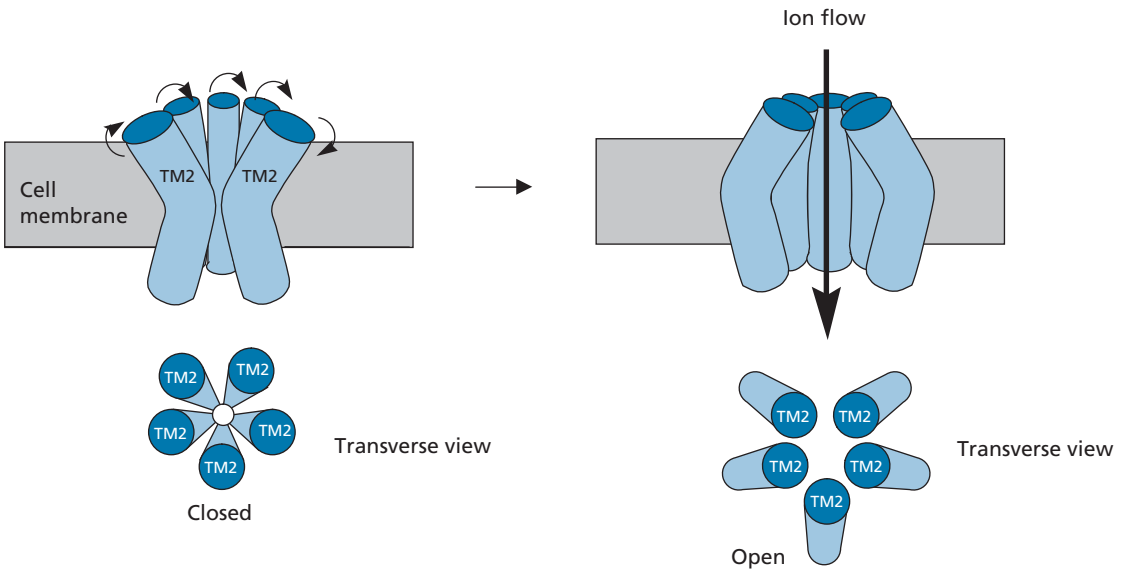


FIGURE 4.13 Opening of the 'lock gate' in an ion channel.

KEY POINTS

- Receptors controlling ion channels are an integral part of the ion channel. Binding of a messenger induces a change in shape, which results in the rapid opening of the ion channel.
- Receptors controlling ion channels are called ligand-gated ion channel receptors. They consist of five protein subunits with the receptor binding site being present on one or more of the subunits.
- Binding of a neurotransmitter to an ion channel receptor causes a conformational change in the protein subunits such that the second transmembrane domain of each subunit rotates to open the channel.

4.7 G-protein-coupled receptors

4.7.1 General principles

The **G-protein-coupled receptors** are some of the most important drug targets in medicinal chemistry. Indeed, some 30% of all drugs on the market act by binding to these receptors. In general, they are activated by hormones and slow-acting neurotransmitters. They include the **muscarinic receptor** (section 22.11), **adrenergic receptors** (section 23.2), and **opioid receptors** (section 24.4). The response from activated G-protein-coupled receptors is measured in seconds. This is slower than the response of ion channels, but faster than the response of kinase-linked receptors (section 4.8), which takes a matter of minutes. There are a large number of different G-protein-coupled receptors interacting with important neurotransmitters, such as acetylcholine, dopamine, histamine, serotonin, glutamate, and noradrenaline. Other G-protein-coupled receptors are activated by peptide and protein hormones, such as the enkephalins and endorphins.

G-protein-coupled receptors are membrane-bound proteins that are responsible for activating proteins called **G-proteins** (Fig. 4.14). These latter proteins act as **signal proteins** because they are capable of activating or deactivating membrane-bound enzymes (sections 5.1–5.2). Consequently, activation of the receptor by a chemical messenger influences the reactions that take place within the cell.

The receptor protein is embedded within the membrane, with the binding site for the chemical messenger exposed on the outer surface. On the inner surface, there is another binding site which is normally closed (Fig. 4.14, frame 1). When the chemical messenger binds to its binding site, the receptor protein changes shape, opening up the binding site on the inner surface. This new binding site is recognized by the G-protein, which then binds (Fig. 4.14, frame 2). The G-protein is attached to the inner surface of the cell membrane and is made up of three protein subunits, but once it binds to the receptor the complex is destabilized and fragments to a monomer and a dimer (Fig. 4.14, frame 3). These then interact with membrane-bound enzymes to continue the signal transduction process (sections 5.1–5.3).

There are several different G-proteins, which are recognized by different types of receptor. Some of the activated subunits from these G-proteins have an inhibitory effect on a membrane-bound enzyme, while others have a stimulatory effect. Nevertheless, the mechanism by which the G-protein is activated by fragmentation is the same.

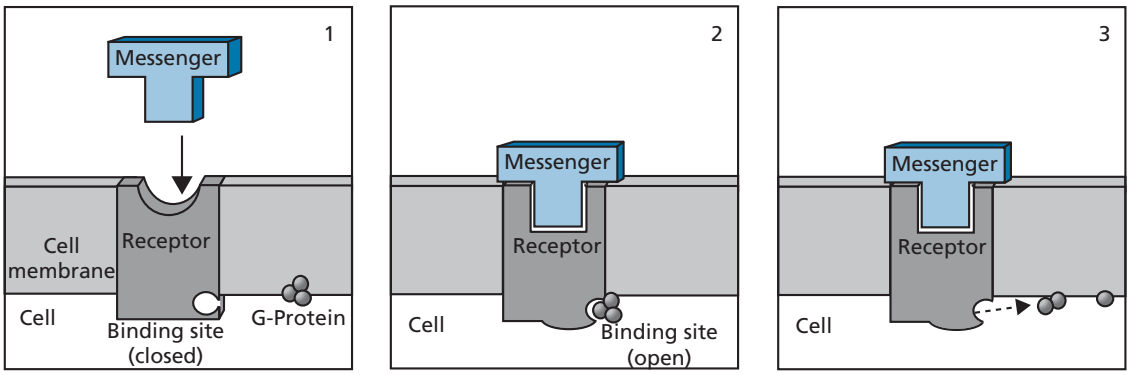


FIGURE 4.14 Activation of a G-protein-coupled receptor and G-protein.

There is a substantial amplification of the signal in this process, as one activated receptor activates several G-proteins.

4.7.2 Structure

The G-protein-coupled receptors fold up within the cell membrane such that the protein chain winds back and forth through the cell membrane seven times (Fig. 4.15). Each of the seven transmembrane sections is hydrophobic and helical in shape, and it is usual to assign these helices with roman numerals (I, II, etc.) starting from the *N*-terminus of the protein. Owing to the number of transmembrane regions, the G-proteins are also called **7-TM receptors**. The binding site for the G-protein is situated on the intracellular side of the protein and involves part of the *C*-terminal chain, as well as part of the variable intracellular loop (so called because the length of this loop varies between different types of receptor). As one might expect, the binding site for the neurotransmitter or hormone messenger is on the extracellular portion of the protein. The exact position of the binding site varies from receptor to receptor. For example, the binding site for the adrenergic receptor is in a deep binding pocket between the transmembrane helices, whereas the binding site for the glutamate receptor involves the *N*-terminal chain and is situated above the surface of the cell membrane.

4.7.3 The rhodopsin-like family of G-protein-coupled receptors

The G-protein-coupled receptors include the receptors for some of the best-known chemical messengers in medicinal chemistry (e.g. glutamic acid, GABA, noradrenaline, dopamine, acetylcholine, serotonin, prostaglandins, adenosine, endogenous opioids, angiotensin, bradykinin, and thrombin). Considering the structural variety of the chemical messengers involved, it is remarkable that the overall structures of the G-protein-coupled receptors

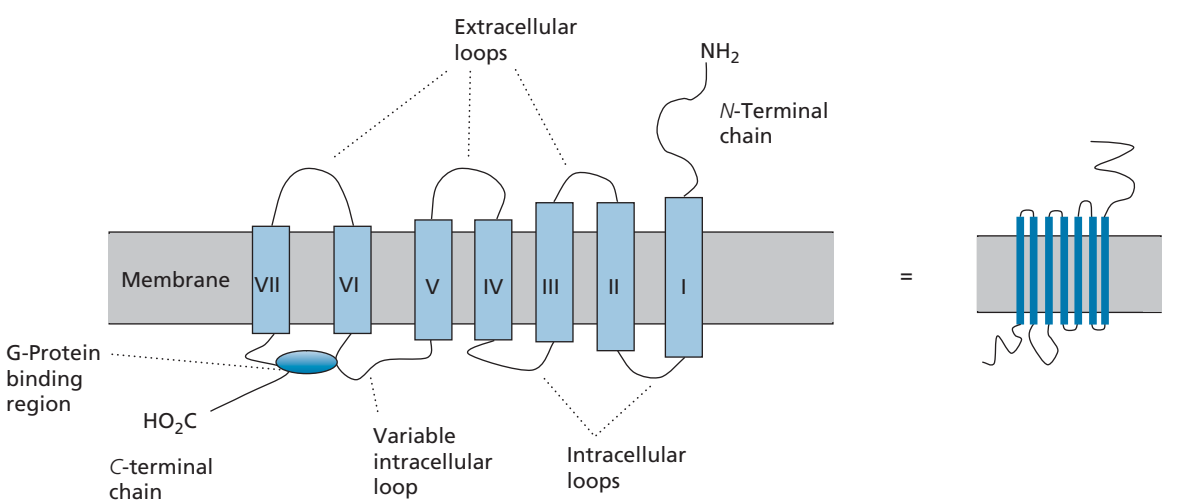


FIGURE 4.15 Structure of G-protein-coupled receptors.

are so similar. Nevertheless, despite their similar overall structure, the amino acid sequences of the receptors vary quite significantly. This implies that these receptors have evolved over millions of years from an ancient common ancestral protein. Comparing the amino acid sequences of the receptors allows us to construct an evolutionary tree and to group the receptors of this superfamily into various sub-families, which are defined as class A (rhodopsin-like receptors), class B (secretin-like receptors), and class C (metabotropic glutamate-like and pheromone receptors). The most important of these, as far as medicinal chemistry is concerned, is the rhodopsin-like family—so called because the first receptor of this family to be studied in detail was the rhodopsin receptor itself, a receptor involved in the visual process. A study of the evolutionary tree of rhodopsin-like receptors throws up some interesting observations (Fig. 4.16).

First of all, the evolutionary tree illustrates the similarity between different kinds of receptors based on their relative positions on the tree. Thus, the muscarinic, α-adrenergic, β-adrenergic, histamine, and dopamine receptors have evolved from a common branch of the evolutionary tree and have greater similarity to each other than to any receptors arising from an earlier evolutionary branch (e.g. the **angiotensin receptor**). Such receptor similarity may prove a problem in medicinal chemistry. Although the receptors are distinguished by different neurotransmitters or hormones in the body, a drug may not manage to make that distinction. Therefore, it is important to ensure that any new drug aimed at one kind of receptor (e.g. the dopamine receptor) does not interact with a similar kind of receptor (e.g. the muscarinic receptor).

Receptors have further evolved to give receptor *types* and *subtypes* which recognize the same chemical messenger, but are structurally different. For example, there are two types of adrenergic receptor (α and β), each of which has various subtypes (α_1, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3). There are two types of cholinergic receptor—nicotinic (an ion channel receptor) and muscarinic (a 7-TM receptor). Five subtypes of the muscarinic cholinergic receptor have been identified.

The existence of receptor subtypes allows the possibility of designing drugs that are selective for one receptor subtype over another. This is important, because one receptor subtype may be prevalent in one part of the body (e.g. the gut), while a different receptor subtype is prevalent in another part (e.g. the heart). Therefore, a drug that is designed to interact selectively with the receptor subtype in the gut is less likely to have side effects on the heart. Even if the different receptor subtypes are present in the same part of the body, it is still important to make drugs as selective as possible because different receptor subtypes frequently activate different signalling systems, leading to different biological results.

A closer study of the evolutionary tree reveals some curious facts about the origins of receptor subtypes. As one might expect, various receptor subtypes have diverged from a common evolutionary branch (e.g. the dopamine subtypes D2, D3, D4). This is known as **divergent evolution** and there should be close structural similarity between these subtypes. However, receptor subtypes are also found in separate branches of the tree. For example, the dopamine receptor subtypes ($D1_A$, $D1_B$, and D5) have developed from a different evolutionary branch. In other words, the ability of a receptor to bind dopamine has developed in different evolutionary branches—an example of **convergent evolution**.

Consequently, there may sometimes be greater similarities between receptors which bind different ligands but which have evolved from the same branch of the tree

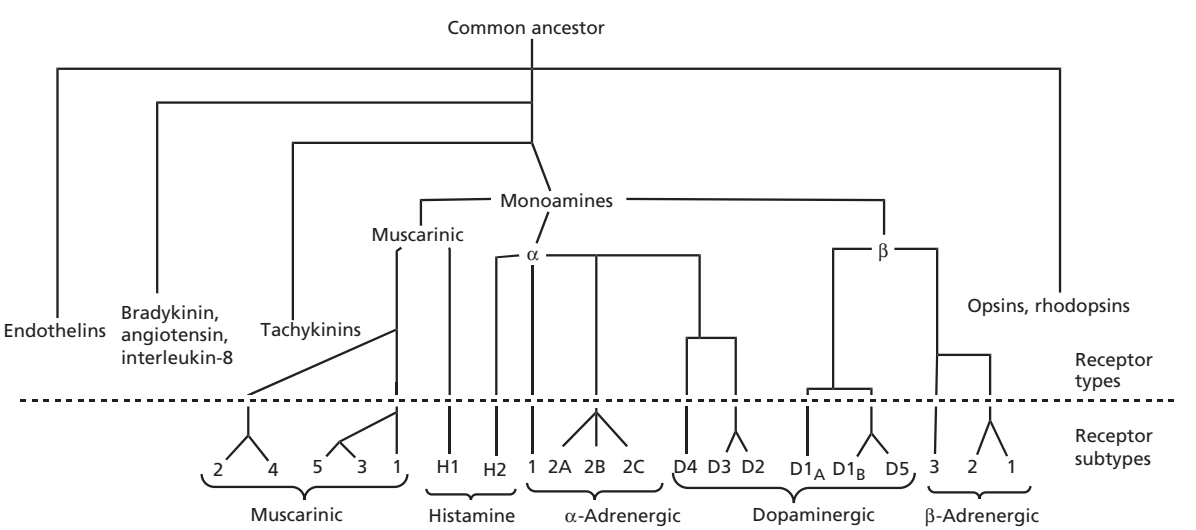


FIGURE 4.16 Evolutionary tree of G-protein-coupled receptors.

than there are between the various subtypes of receptors which bind the same ligand. For example, the histamine H_1 receptor resembles a muscarinic receptor more closely than it does the histamine H_2 receptor. Again, this has important consequences in drug design because there is an increased possibility that a drug aimed at a muscarinic receptor may also interact with a histamine H_1 receptor and lead to unwanted side effects.

As these receptors are membrane bound, it is not easy to crystallize them for X-ray crystallographic studies. However, the X-ray crystal structures of the β_2 and β_1 adrenoceptors have now been determined.

4.7.4 Dimerization of G-coupled receptors

There is strong evidence that some G-coupled receptors can exist as dimeric structures containing identical or different types of receptor—homodimers or heterodimers respectively. The presence of these receptor dimers appears to vary between different tissues and this has important consequences for drug design. An agent that is selective for one type of receptor would not normally affect other types. However, if receptor heterodimers are present, a 'communication' is possible between the component receptors such that an agent interacting with one half of the dimer may affect the activity of the other half. This is discussed further in section 24.9 with respect to opioid receptors.

KEY POINTS

- G-protein-coupled receptors activate signal proteins called G-proteins. Binding of a messenger results in the opening of a binding site for the signal protein. The latter binds and fragments, with one of the subunits departing to activate a membrane-bound enzyme.
- The G-protein-coupled receptors are membrane-bound proteins with seven transmembrane sections. The *C*-terminal chain lies within the cell and the *N*-terminal chain is extracellular.
- The location of the binding site differs between different G-protein-coupled receptors.
- The rhodopsin-like family of G-protein-coupled receptors includes many receptors that are targets for currently important drugs.
- Receptor types and subtypes recognize the same chemical messenger, but have structural differences, making it possible to design drugs that are selective for one type (or subtype) of receptor over another.
- Receptor subtypes can arise from divergent or convergent evolution.
- It is possible for some G-protein coupled receptors to exist as dimeric structures.

4.8 Kinase-linked receptors

4.8.1 General principles

Kinase-linked receptors are a superfamily of receptors which activate enzymes directly and do not require a G-protein (Fig. 4.17). **Tyrosine kinase receptors** are important examples of kinase-linked receptors and are proving to be highly important targets for novel anticancer drugs (section 21.6.2). In these structures, the protein concerned plays the dual role of receptor and enzyme. The receptor protein is embedded within the cell membrane, with part of its structure exposed on the outer surface of the cell and part exposed on the inner surface. The outer surface contains the binding site for the chemical messenger and the inner surface has an active site that is closed in the resting state. When a chemical messenger binds to the receptor it causes the protein to change shape. This results in the active site being opened up, allowing the protein to act as an enzyme within the cell. The reaction that is catalysed is a phosphorylation reaction where tyrosine residues on

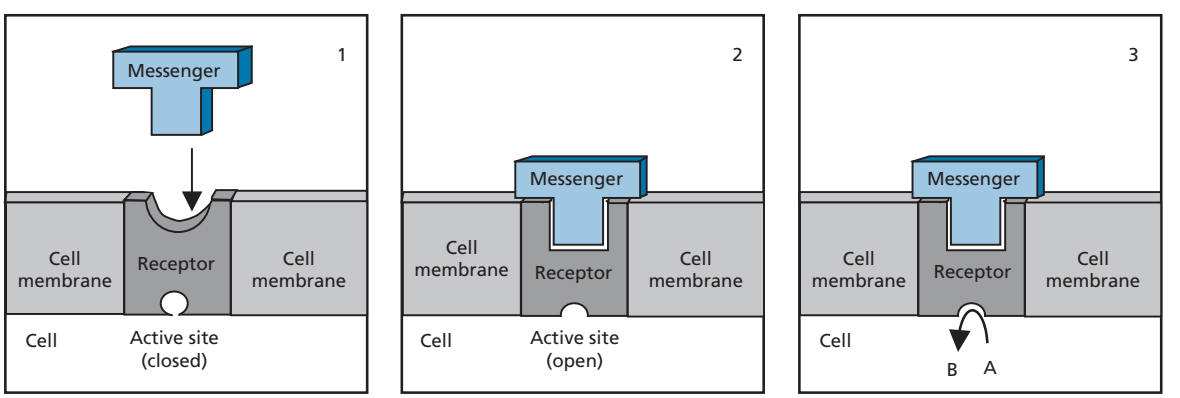


FIGURE 4.17 Enzyme activation.

a protein substrate are phosphorylated. An enzyme that catalyses phosphorylation reactions is known as a kinase enzyme and so the protein is referred to as a tyrosine kinase receptor. ATP is required as a cofactor to provide the necessary phosphate group. The active site remains open for as long as the messenger molecule is bound to the receptor, and so several phosphorylation reactions can occur, resulting in an amplification of the signal. A curiosity of this enzyme-catalysed reaction is that the substrate for the reaction is the receptor itself. This is explained more fully in section 4.8.3.

The kinase-linked receptors are activated by a large number of polypeptide hormones, growth factors, and cytokines. Loss of function of these receptors can lead to developmental defects or hormone resistance. Overexpression can result in malignant growth disorders.

4.8.2 Structure of tyrosine kinase receptors

The basic structure of a tyrosine kinase receptor consists of a single extracellular region (the *N*-terminal chain) that includes the binding site for the chemical messenger, a single hydrophobic region that traverses the membrane as an α-helix of seven turns (just sufficient to traverse the membrane), and a *C*-terminal chain on the inside of the cell membrane (Fig. 4.18). The *C*-terminal region contains the catalytic binding site. Examples of tyrosine kinase receptors include the receptor for **insulin,** and receptors for various **cytokines** and **growth factors**.

4.8.3 Activation mechanism for tyrosine kinase receptors

A specific example of a tyrosine kinase receptor is the receptor for a hormone called **epidermal growth factor** (EGF). EGF is a **bivalent ligand** which can bind to two receptors at the same time. This results in **receptor dimerization**, as well as activation of enzymatic activity. The dimerization process is important because the active site on each half of the receptor dimer catalyses the phosphorylation of accessible tyrosine residues on the other half (Fig. 4.19). If dimerization did not occur, no phosphorylation would take place. Note that these phosphorylations occur on the intracellular portion of the receptor protein chain. The relevance of these phosphorylation reactions will be explained in section 5.4.1. The important point to grasp at this stage is that an external chemical messenger has managed to convey its message to the interior of the cell without itself being altered or having to enter the cell.

Dimerization and auto-phosphorylation are common themes for receptors in this family. However, some of the receptors in this family already exist as dimers or tetramers, and only require binding of the ligand. For example, the **insulin** receptor is a heterotetrameric complex (Fig. 4.20).

4.8.4 Tyrosine kinase-linked receptors

Some kinase receptors bind ligands and dimerize in a similar fashion to the ones described above, but do not have inherent catalytic activity in their *C*-terminal chain. However, once they have dimerized, they can bind and activate a tyrosine kinase enzyme from the cytoplasm. The **growth hormone** (GH) receptor is an example of this type of receptor and is classified as a tyrosine kinase-linked receptor (Fig. 4.21).

KEY POINTS

- Kinase-linked receptors are receptors which are directly linked to kinase enzymes. Messenger binding results in the opening of the kinase-active site, allowing a catalytic reaction to take place.
- Tyrosine kinase receptors have an extracellular binding site for a chemical messenger and an intracellular enzymatic

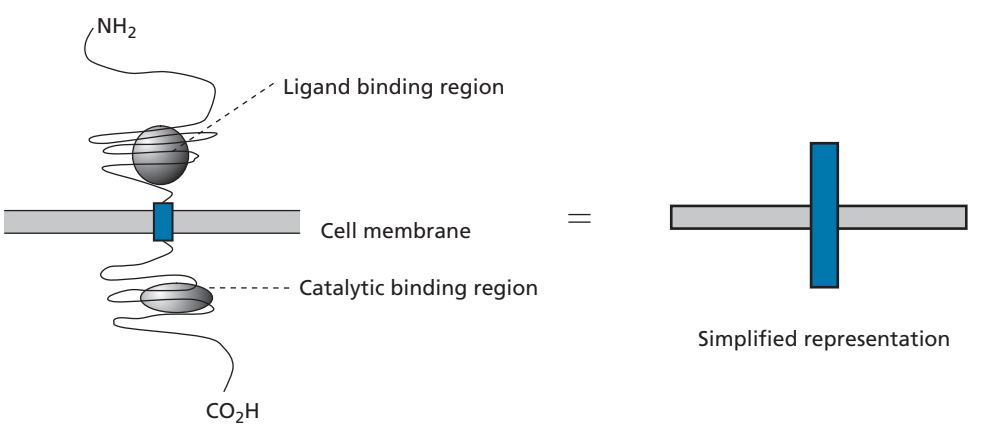


FIGURE 4.18 Structure of tyrosine kinase receptors.

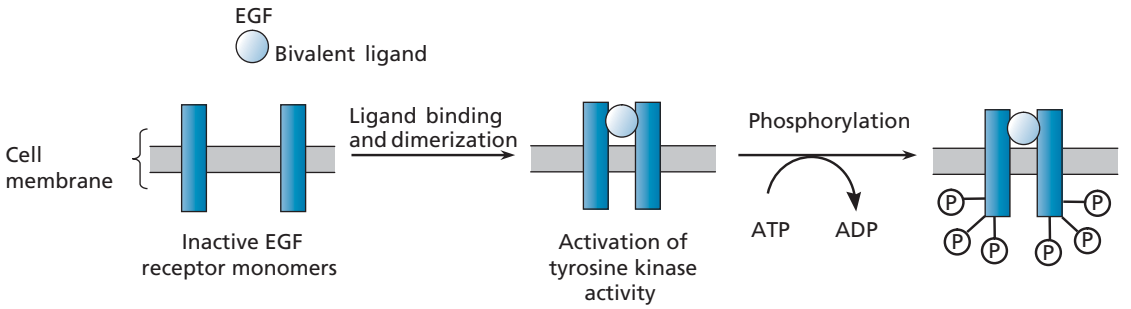


FIGURE 4.19 Activation mechanism for the epidermal growth factor (EGF) receptor.

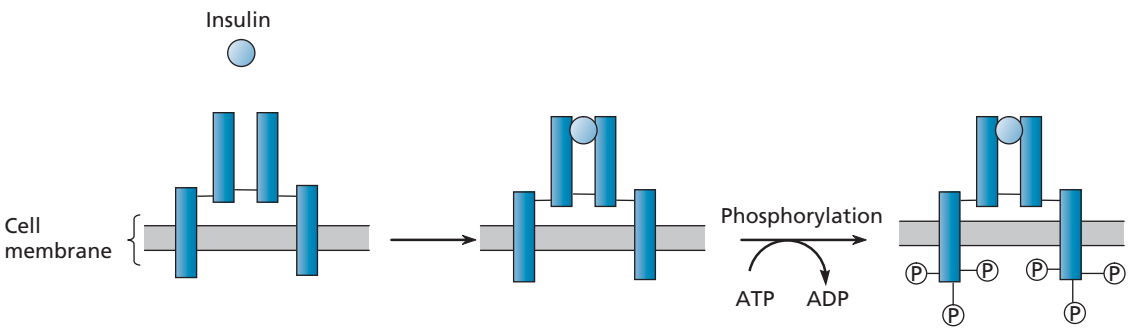


FIGURE 4.20 Ligand binding and activation of the insulin receptor.

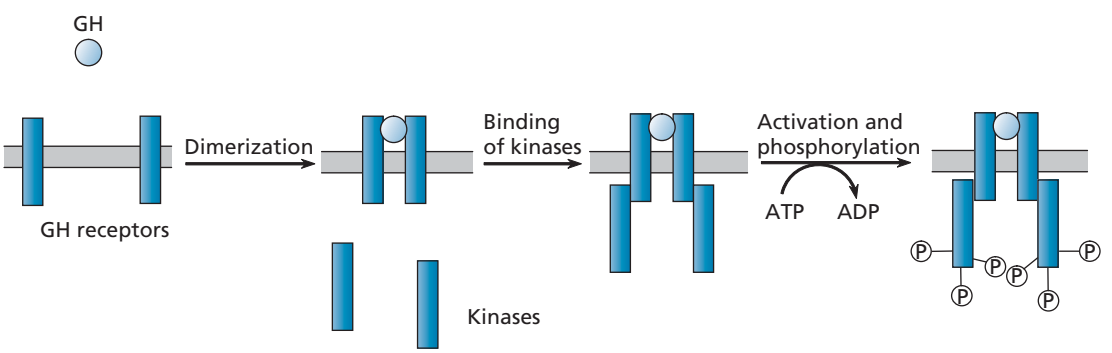


FIGURE 4.21 Activation of the growth hormone (GH) receptor.

active site which catalyses the phosphorylation of tyrosine residues in protein substrates.

- Ligand binding to the epidermal growth factor (EGF) receptor results in dimerization and opening of the active sites. The active site on one half of the dimer catalyses the phosphorylation of tyrosine residues present on the *C*-terminal chain of the other half.
- The insulin receptor is a preformed heterotetrameric structure which acts as a tyrosine kinase receptor.
- The growth hormone receptor dimerizes on binding its ligand, then binds and activates tyrosine kinase enzymes from the cytoplasm.

4.9 Intracellular receptors

Not all receptors are located in the cell membrane. Some receptors are within the cell and are defined as intracellular receptors. There are about 50 members of this group and they are particularly important in directly regulating gene transcription. As a result, they are often called **nuclear hormone receptors** or **nuclear transcription factors.** The chemical messengers for these receptors include steroid hormones, thyroid hormones, and retinoids. In all these cases, the messenger has to pass through the cell membrane in order to reach its receptor so it has to be hydrophobic in nature. The response time

resulting from the activation of the intracellular receptors is measured in hours or days, and is much slower than the response times of the membrane-bound receptors.

The intracellular receptors all have similar general structures. They consist of a single protein containing a ligand binding site at the *C*-terminus and a binding region for DNA near the centre (Fig. 4.22). The DNA binding region contains nine cysteine residues, eight of which are involved in binding two zinc ions. The zinc ions play a crucial role in stabilizing and determining the conformation of the DNA binding region. As a result, the stretches of protein concerned are called the **zinc finger domains.** The DNA binding region for each receptor can identify particular nucleotide sequences in DNA. For example, the zinc finger domains of the **estrogen receptor** recognize the sequence 5′-AGGTCA-3′, where A, G, C, and T are adenine, guanine, cytosine, and thymine.

The mechanism by which intracellular receptors work is also very similar (Fig. 4.23). Once the chemical messenger (ligand) has crossed the cell membrane, it seeks out its receptor and binds to it at the ligand binding site. An induced fit takes place which causes the receptor to change shape. This, in turn, leads to a dimerization of the ligand–receptor complex. The dimer then binds to a protein called a **co-activator** and, finally, the whole complex binds to a particular region of the cell's DNA. As there are two receptors in the complex and two DNA binding regions, the complex recognizes two identical sequences of nucleotides in the DNA separated by a short distance. For example, the estrogen ligand–receptor dimer binds to a nucleotide sequence of 5′-AGGTCANNNTGACCT-3′ where N can be any nucleic acid base. Depending on the complex involved, binding of the complex to DNA either triggers or inhibits the start of transcription, and affects the eventual synthesis of a protein.

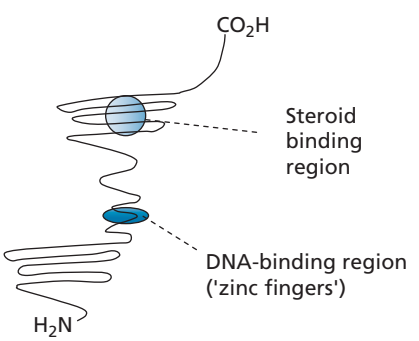


FIGURE 4.22 Structure of intracellular receptors.

4.10 Regulation of receptor activity

The role of allosteric binding sites in regulating the activity of enzymes was covered in section 3.6. Allosteric binding sites also play a role in regulating or modulating the activity of various receptors. These include ligand-gated ion channels, such as the nicotinic and the γ-aminobutyric acid receptors, and several G-protein-coupled receptors, such as the muscarinic, adenosine, and dopamine receptors. Structures that interact with these sites are called **allosteric modulators** and can either enhance or decrease the effect of the chemical messenger on the receptor (sections 8.2.7 and 8.3.2).

4.11 Genetic polymorphism and receptors

Genetic polymorphism was discussed in section 3.5.6 with respect to enzymes. Polymorphism is also responsible for receptors having subtle differences in structure and activity between individuals. In some cases, this can lead to diseases such as cancer (section 21.1.3).

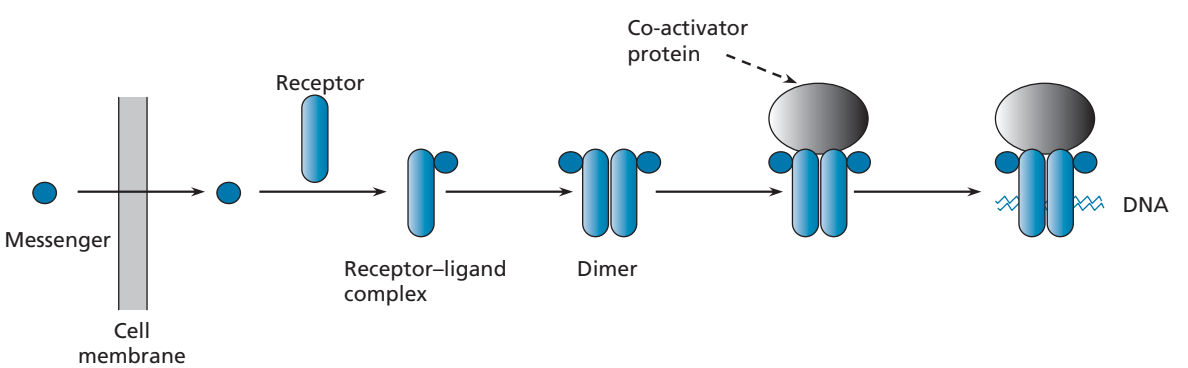


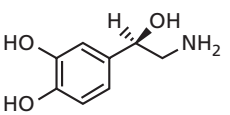
FIGURE 4.23 From messenger to control of gene transcription.

KEY NOTES

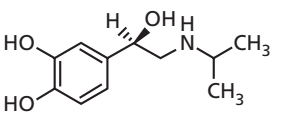
- Intracellular receptors are located within the cell and are important in controlling transcription.
- The chemical messengers for intracellular receptors must be sufficiently hydrophobic to pass through the cell membrane.
- The binding of a ligand with an intracellular receptor results in dimerization and the formation of a transcription factor complex which binds to a specific nucleotide sequence on DNA.

QUESTIONS

1. Explain the distinction between a binding site and a binding region.
2. Consider the structures of the neurotransmitters shown in Fig. 4.3 and suggest what type of binding interactions could be involved in binding them to a receptor binding site. Identify possible amino acids in the binding site which could take part in each of these binding interactions.
3. There are two main types of adrenergic receptor: the α and β–adrenoceptors. Noradrenaline shows slight selectivity for the α-receptor, whereas isoprenaline shows selectivity for the β-adrenoceptor. Adrenaline shows no selectivity and binds equally well to both the α- and β-adrenoceptors. Suggest an explanation for these differences in selectivity.



Noradrenaline



Isoprenaline

4. Suggest why the transmembrane regions of many membrane-bound proteins are α-helices.

FURTHER READING

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