# 8 Receptors as drug targets

## 8.1 Introduction

The structures and functions of various receptors were described in chapter 4. Receptors and their chemical messengers are crucial to the communication systems of the body. Such communication is clearly essential to the normal workings of the body, and when it goes wrong, a huge variety of ailments can arise, such as depression, heart problems, schizophrenia, and muscle fatigue to name just a few. What sort of things *could* go wrong though?

One problem would be if too many chemical messengers were released. The target cell could (metaphorically) start to overheat. Alternatively, if too few messengers were sent out, the cell could become sluggish. It is at this point that drugs can play a role by either acting as replacement messengers, or blocking receptors from receiving their natural messengers. Drugs that mimic the natural messengers and activate receptors are known as **agonists**. Drugs that block receptors are known as **antagonists**. These compounds still bind to the receptor, but they do not activate it. However, since they are bound, they prevent the natural messenger from binding.

What determines whether a drug acts as an agonist or an antagonist, and is it possible to predict whether a new drug will act as one or the other? To answer these questions, we have to move down to the molecular level and consider what happens when a small molecule such as a drug or a neurotransmitter interacts with a receptor protein.

In sections 4.4–4.5, we looked at a hypothetical receptor and neurotransmitter. We saw that a chemical messenger caused the receptor to change shape—a process known as an induced fit. It is this induced fit which activates the receptor and leads to the 'domino' effect of signal transduction—the method by which the message carried by the chemical messenger is transferred into the cell (chapters 4 and 5).

## 8.2 The design of agonists

We are now at the stage of understanding how drugs might be designed in such a way that they mimic the natural neurotransmitters. Assuming that we know what binding regions are present in the receptor site and where they are, we can design drugs to interact with the receptor in the same way. Let us look at this more closely and consider the following requirements in turn.

- The drug must have the correct binding groups.
- The drug must have these binding groups correctly positioned.
- The drug must be the right size for the binding site.

### 8.2.1 Binding groups

If we know the structure of the natural chemical messenger, and can identify the functional groups that are important for binding it to its receptor, then we might reasonably predict which of a series of molecules would interact with the receptor in the same way. For example, consider the hypothetical neurotransmitter shown in Fig 8.1. The important binding groups are indicated in blue—an aromatic ring, alcohol and ammonium ion. These interact with the binding site through van der Waals interactions, hydrogen bonding and ionic bonding respectively. Consider now the other structures in Fig. 8.1. They all look different, but they all contain functional groups which could interact in the same way. Therefore, they may well be potential agonists which will activate the receptor.

Consider now the structures in Fig. 8.2. They lack one or more of the required binding groups and might therefore be expected to have poor activity. We would expect them to drift into the receptor site and then drift back out again, binding only weakly if at all.

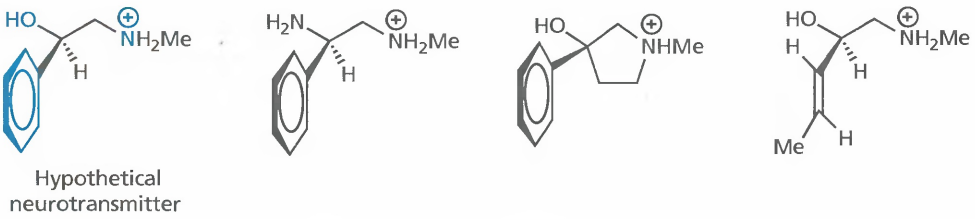


**FIGURE 8.1** A hypothetical neurotransmitter and possible agonists (binding groups shown in blue).

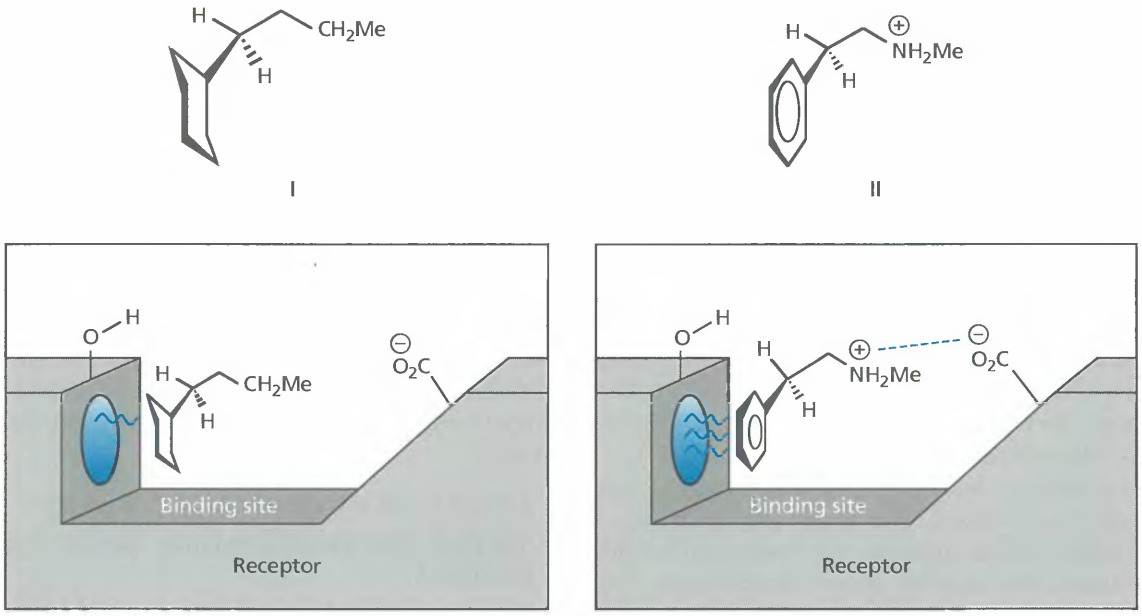


**FIGURE 8.2** The weaker binding to the hypothetical receptor by structures that possess fewer than the required binding groups.

Of course, we are making an assumption here; mainly that all three binding groups are essential. It might be argued that a compound such as structure II in Fig. 8.2 might be effective even though it lacks a suitable hydrogen bonding group. Why, for example, could it not bind initially by van der Waals interactions alone and then alter the shape of the receptor protein via ionic bonding?

In fact, this seems unlikely when we consider that neurotransmitters appear to bind, pass on their message and then leave the binding site relatively quickly. In order to do that, there must be a fine balance in the binding interactions between the receptor and the neurotransmitter. They must be strong enough to bind the neurotransmitter effectively such that the receptor changes shape. However, the binding interactions cannot be too strong, or else the neurotransmitter would not be able to leave and the receptor would not be able to return to its original shape. Therefore, it is reasonable to assume that a neurotransmitter needs all of its binding interactions to be effective. The lack of even one of these interactions would lead to a significant loss in activity.

## 8.2.2 Position of the binding groups

The molecule may have the correct binding groups, but if they are in the wrong relative positions, they will not be able to form bonds at the same time. As a result, bonding would be too weak to be effective.

A molecule such as the one shown in Fig. 8.3 obviously has one of its binding groups (the hydroxyl group) in the wrong position, but there are more subtle examples of molecules that do not have the correct arrangement of binding groups. For example, the mirror image of our hypothetical neurotransmitter would not bind strongly to the binding site (Fig. 8.4). The structure has the same formula and the same constitutional structure as our original structure. It will have the same physical properties and undergo the same chemical reactions, but it is not the same shape. It is a non-superimposable mirror

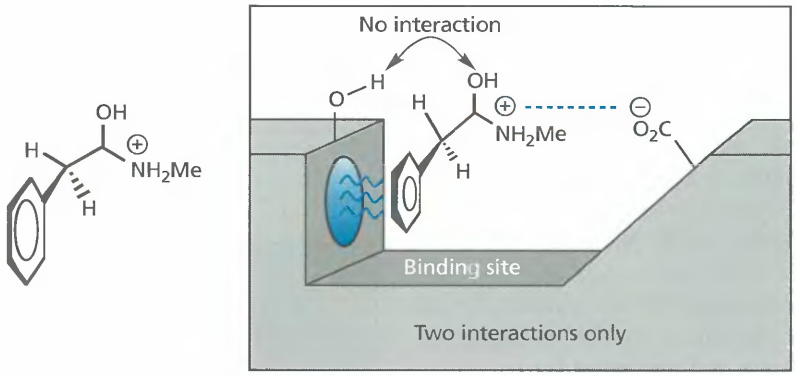


**FIGURE 8.3** The weaker binding to the hypothetical receptor by a molecule containing binding groups in incorrect positions.

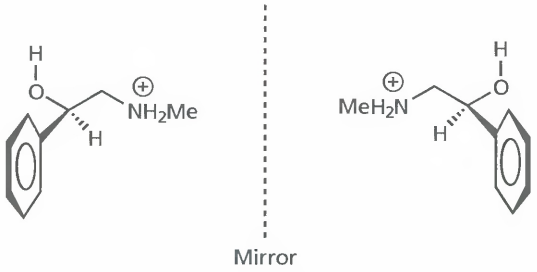


**FIGURE 8.4** Mirror image of a hypothetical neurotransmitter.

image and it cannot interact with all the binding regions of the receptor binding site at the same time (Fig. 8.5).

Compounds which have non-superimposable mirror images are termed **chiral** or **asymmetric**. There are only two detectable differences between the two mirror images (or **enantiomers**) of a chiral compound. They rotate plane polarized light in opposite directions, and they interact differently with other chiral systems such as enzymes. This has very important consequences for the pharmaceutical industry.

Pharmaceutical agents are usually synthesized from simple starting materials using simple achiral (symmetrical) chemical reagents. These reagents are incapable of distinguishing between the two mirror images of a chiral compound. As a result, most chiral drugs used to be synthesized as a mixture of both mirror images—a **racemate**. However, we have seen from our own simple example that only one of these enantiomers is going to interact properly with a target receptor. What happens to the other enantiomer?

At best, it floats about in the body doing nothing. At worst, it interacts with a totally different receptor and results in an undesired side effect. Even if the 'wrong' enantiomer does not do any harm, it seems to be a great waste of time, money and effort to synthesize drugs that are only going to be 50% efficient. That is why one of the biggest areas of chemical research in

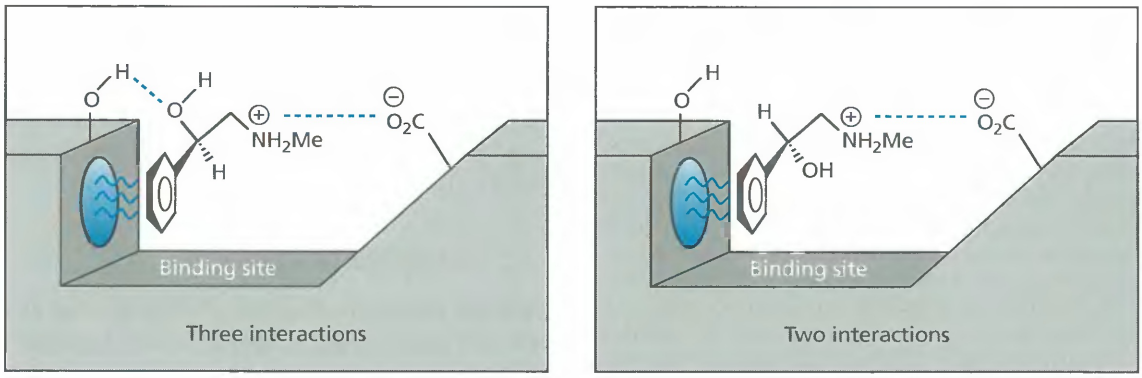


**FIGURE 8.5** A comparison of how the hypothetical neurotransmitter and its mirror image interact with the receptor binding site.

recent years has been in **asymmetric synthesis**—the selective synthesis of a single enantiomer of a chiral compound.

Of course, nature has been at it for millions of years. Nature has chosen to work predominantly with the 'left-handed' enantiomer of amino acids,[1] so enzymes (made up of left-handed amino acids) are also present as a single mirror image and therefore catalyse **enantiospecific** reactions—reactions which give only one enantiomer. This also means that the enantiomers of asymmetric enzyme inhibitors can be distinguished by the target enzyme so that one enantiomer is more potent than the other.

The importance of having binding groups in the correct position has led medicinal chemists to design drugs based on what is considered to be the important **pharmacophore** of the messenger molecule. In this approach, it is assumed that the correct positioning of the binding groups is what decides whether the drug will act as a messenger or not, and that the rest of the molecule serves only to hold the groups in those positions. Therefore, the activity of apparently disparate structures at a receptor can be explained if they all contain the correct binding groups at the correct positions. Totally novel structures or molecular frameworks could then be designed to obey this rule, leading to a new series of drugs. There is, however, a limiting factor to this, which will now be discussed.

## 8.2.3 Size and shape

It is possible for a compound to have the correct binding groups in the correct positions, and yet fail to interact effectively if it has the wrong size or shape. As an example, consider the structure shown in Fig. 8.6 as a possible candidate for our hypothetical receptor.

The structure has a *meta*-methyl group on the aromatic ring and a long alkyl chain attached to the nitrogen atom. Both of these features would prevent this molecule from binding effectively to the binding site shown.

The *meta*-methyl group acts as a buffer and prevents the structure from sinking deep enough into the binding site for effective binding. Furthermore, the long alkyl chain on the nitrogen atom makes that part of the molecule too long for the space available to it. A thorough understanding of the space available in the binding site is therefore necessary when designing drugs to fit it.

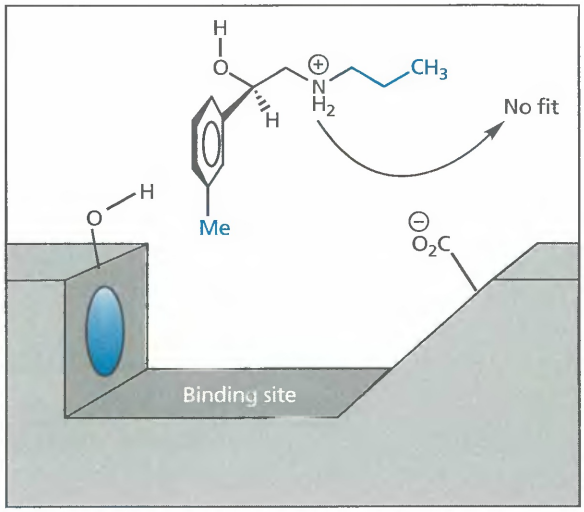


**FIGURE 8.6** Failed interaction of a structure with a binding site, because of steric factors.

## 8.2.4 Pharmacodynamics and pharmacokinetics

The study of how molecules interact with targets such as receptors or enzymes is called pharmacodynamics. Such studies can typically be carried out on the pure target protein, or on isolated cells or tissues which bear the target protein (***in vitro* studies**), but it is important to appreciate that designing a drug to interact effectively with a protein *in vitro* does not guarantee a clinically useful drug. Studies should also be carried out concurrently to ensure that promising-looking drugs are active in whole organisms (***in vivo* studies**). This is a field known as pharmacokinetics and is covered in chapters 11 and 14. It is also important to identify at an early stage whether the structures being studied might be prone to toxic or unacceptable side effects, such that time is not wasted taking a candidate drug all the way to clinical trials, only for it to be rejected (Box 12.3).

## 8.2.5 Examples of agonists

There are numerous examples of drugs that act as agonists at various target receptors. In this textbook, you will find a description of cholinergic agonists which are used in the treatment of glaucoma and myasthenia gravis (section 22.10). There is a description of the adrenergic

[1] Naturally occurring asymmetric amino acids exist in mammals as the one enantiomer, termed the L-enantiomer. This terminology is historical, and defines the absolute configuration of the asymmetric carbon present at the head-group of the amino acid. The current terminology for asymmetric centres is to define them as R or S according to a set of rules known as the Cahn–Ingold–Prelog rules. The L-amino acids exist as the (S)-configuration (except for cysteine which is R), but the older terminology still dominates here. Experimentally, the L-amino acids are found to rotate plane-polarized light anticlockwise or to the left. It should be noted that D-amino acids can occur naturally in bacteria (see for example section 19.5.5).

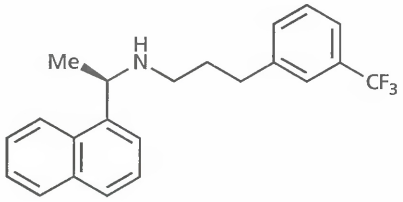


**FIGURE 8.7** Cinacalcet.

agonists used as anti-asthmatic agents in section 23.10.3, while chapter 24 covers the opioid analgesics which act as agonists.

Other examples of agonists used in the clinic are dopamine agonists used in the treatment of Parkinson's disease, and serotonin agonists used in the treatment of migraine. Agonists designed to act on the oestrogen receptor are used as contraceptives. There are many, many more examples.

### 8.2.6 Allosteric modulators

Some drugs have an indirect agonist effect by acting as allosteric modulators. By binding to an allosteric site on a target receptor, they mimic the action of endogenous modulators and enhance the action of the natural or endogenous chemical messenger (section 4.10). For example, the benzodiazepines used as sleep medicines target the allosteric binding site of the $GABA_A$ receptor. **Cinacalcet** (Fig. 8.7) is used to treat thyroid problems, and is an allosteric modulator for a G-protein-coupled receptor known as the calcium-sensing receptor. **Galantamine** acts as an enzyme inhibitor in the treatment of Alzheimer's disease (section 22.17), but is also an allosteric modulator of the nicotinic receptor.

## 8.3 The design of antagonists

### 8.3.1 Antagonists acting at the binding site

We have seen how it might be possible to design drugs (agonists) to mimic natural neurotransmitters, and how these would be useful in treating a shortage of the natural neurotransmitter. However, suppose that we have too much neurotransmitter operating in the body. How could a drug counteract that? The answer would be to design a drug (an antagonist) that will bind to the binding site, but will not activate the receptor. Since it is bound, it will prevent the normal messenger from binding and activating the receptor.

There are several strategies in designing antagonists, but one way is to design a drug which is the right shape to bind to the receptor binding site, but which either fails to change the shape of the binding site or distorts it in the wrong way. Consider the following scenario.

The compound shown in Fig. 8.8 fits the binding site perfectly and, as a result, does not cause any change of shape. Therefore, there is no biological effect and the binding site is blocked to the natural neurotransmitter.

Another strategy is to find different binding regions within the binding site that are not used by the natural chemical messenger. It has to be remembered that the binding site is bristling with amino acid residues and peptide links, all of which might be capable of interacting with a visiting molecule by different intermolecular binding interactions. In other words, there may be other binding regions present than just those used by the natural messenger (Fig. 8.9). Drugs could be designed to interact with some of these extra binding regions, such that the resultant binding produces a quite different induced fit from that obtained when the natural messenger binds—an induced fit that fails to activate the receptor.

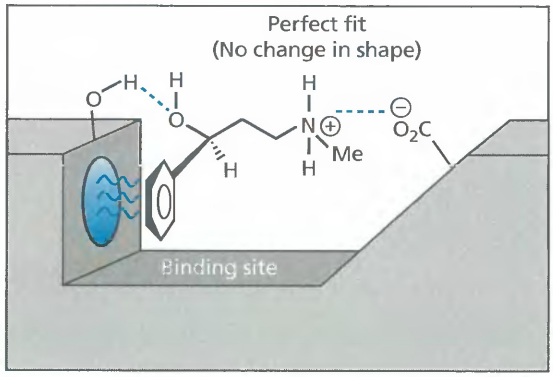


**FIGURE 8.8** Compound acting as an antagonist at the binding site.

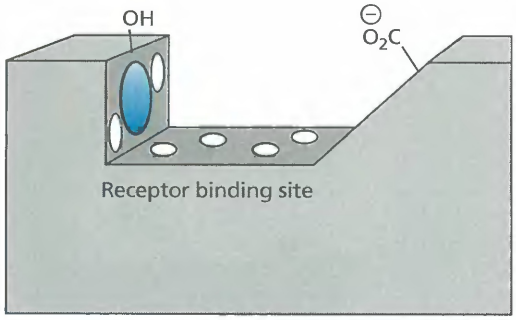


**FIGURE 8.9** The hypothetical binding site showing extra binding regions (in white) that are not used by the natural chemical messenger.

Extra binding regions do not necessarily have to be within the part of the binding site occupied by the natural messenger. It is quite common to find antagonists that are larger than the natural messenger and which access extra binding regions beyond the reach of the usual messenger. Many antagonists are capable of binding to both the normal binding site and these neighbouring regions.

To illustrate this, we will once more consider our hypothetical neurotransmitter and its receptor, but this time we will represent the binding site in a different way, as if we were looking at it from above and drawing a map of where the binding regions are located (Fig. 8.10). This kind of representation is used frequently in order to simplify binding site diagrams, but it is important to appreciate that the binding site is a three-dimensional shape and that the interactions involved are also in three dimensions.

The three important binding regions are still present, but our 'map' shows an extra hydrophobic region which could act as a potential binding region.

Binding of the hypothetical neurotransmitter results in the correct induced fit required for receptor activation (Fig. 8.11). Note that the extra binding region is not within range of the messenger molecule.

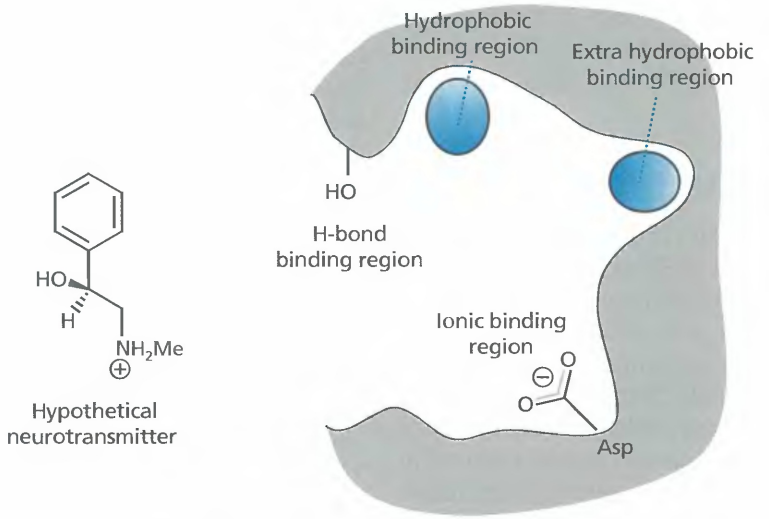


**FIGURE 8.10** 'Map' of the hypothetical binding site.

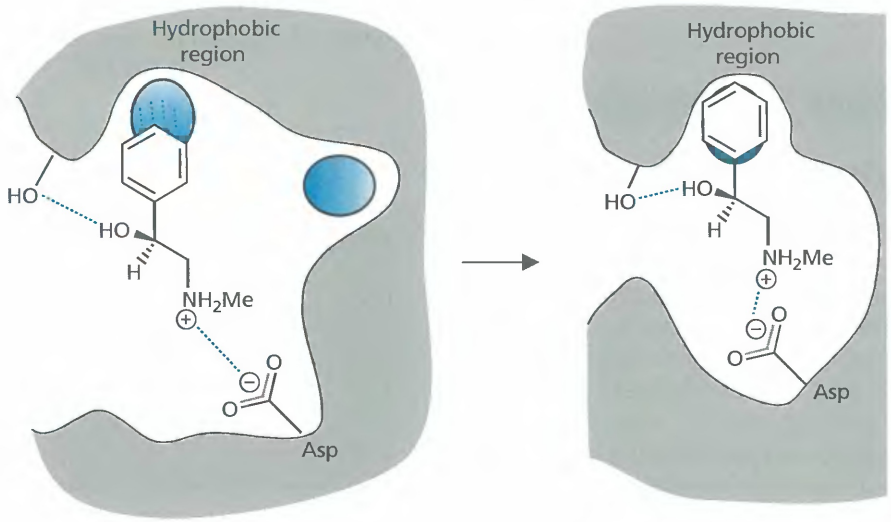


**FIGURE 8.11** Binding of the natural chemical messenger, resulting in an induced fit that activates the receptor.

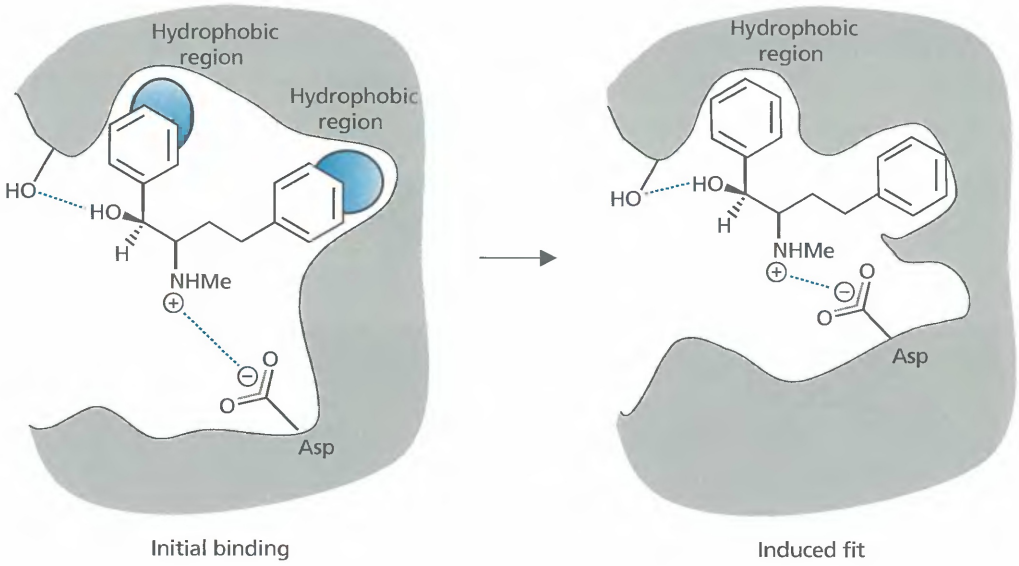


**FIGURE 8.12** Binding of an antagonist leading to a different induced fit.

We could now design a molecule which would bind to all four of these binding regions (Fig. 8.12). This molecule will bind more strongly than the natural messenger due to the extra binding interaction, but it would be expected to produce a different induced fit. Since it is a different induced fit, the receptor will not be activated and the molecule acts as an antagonist since it prevents the normal messenger from binding. Antagonists which bind strongly to a target binding site can be used to label receptors (Box 8.1). Such antagonists are often synthesized with a radioactive isotope incorporated into the structure, so that they can be detected more easily.

To sum up, if we know the shape and make-up of a receptor binding site, then we should be able to design drugs to act as agonists *or* antagonists. Unfortunately, it is not as straightforward as it sounds. Finding the receptor and determining the layout of its binding site is no easy task. In the past the theoretical shape of many receptor sites was hypothesized by synthesizing a large number of compounds and identifying those molecules that fitted and those that did not—a bit like a three-dimensional jigsaw. Nowadays, the use of genetic engineering, X-ray crystallography of protein targets, and computer-based molecular modelling allows a more accurate representation of proteins and their binding sites (chapter 17). This has heralded new approaches to developing new drugs such as *de novo* drug design and structure-based drug design (see also Box 8.2).

There are many examples in this book of antagonists that act at the binding site of a receptor. These include the histamine $H_2$ antagonists used for the treatment of ulcers (chapter 25), the adrenergic antagonists used in cardiovascular medicine (section 23.11.3), serotonin antagonists as potential CNS-active drugs (Case Studies 6 and 7) and the cholinergic antagonists used as neuromuscular blockers (section 22.12.2). Another example is raloxifene, which acts as an antagonist of the oestrogen receptor (Box 8.2). This compound is an example of an antagonist that binds to the same binding regions as the natural ligand, but also binds to an extra binding region.

## BOX 8.1 Antagonists as molecular labels

Antagonists generally bind more strongly to receptors than agonists. As a result, antagonists have been useful in the isolation and identification of specific receptors present in tissues. A useful tactic in this respect is to incorporate a highly reactive chemical group—usually an electrophilic group—into the structure of a powerful antagonist. The electrophilic group will then react with any convenient nucleophilic group on the receptor surface and alkylate it to form a strong covalent bond. The antagonist will then be irreversibly tied to the receptor and can act as a molecular label. One example is tritium-labelled **propylbenzilylcholine mustard**—used to label the **muscarinic cholinergic receptor** (Chapter 19).

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## BOX 8.1 Antagonists as molecular labels *(Continued)*

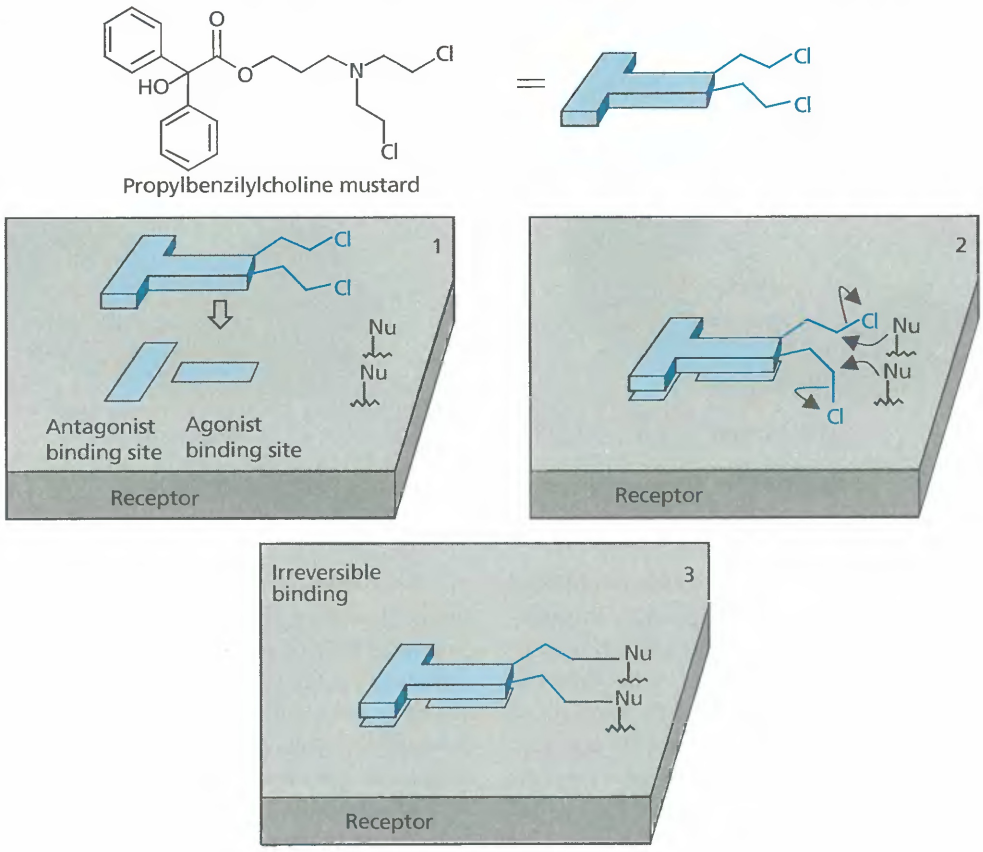



**FIGURE 1** Antagonist used as a molecular label.

## BOX 8.2 Oestradiol and the oestrogen receptor

17β-Oestradiol (spelt estradiol in the USA) is a steroid hormone which affects the growth and development of a number of tissues. It does so by crossing cell membranes and interacting with the binding site of an oestrogen intracellular receptor. Oestradiol uses its alcohol and phenol groups to form hydrogen bonds with three amino acids in the binding site, while the hydrophobic skeleton of the molecule forms van der Waals and hydrophobic interactions with other regions (Fig. 1). The binding pocket is hydrophobic in nature and quite spacious, except for the region where the phenol ring binds. This is a narrow slot and will only accept a planar aromatic ring. Due to these constraints, the binding of oestradiol's phenolic ring determines the orientation for the rest of the molecule.

The binding of oestradiol induces a conformational change in the receptor which sees a helical section known as H12) folding across the binding site like a lid (Fig. 2). This not only seals oestradiol into its binding site, it also exposes a hydrophobic region called the activating function (AF-2) region which acts as a binding site for a coactivator protein. Since dimerization has also taken place, there are two of these regions available and the coactivator binds to both to complete the nuclear transcription factor. This now binds to a specific region of DNA and switches on the transcription of a gene, resulting in the synthesis of a protein.

**Raloxifene** (Fig. 3) is an antagonist of the oestrogen receptor and is used for the treatment of hormone-dependent breast cancer. It is a synthetic agent that binds to the binding site without activating the receptor, and prevents oestradiol from binding. The molecule has two phenol groups, which mimic the phenol and alcohol group of oestradiol. The skeleton is also hydrophobic and matches the hydrophobic character of oestradiol. So why does raloxifene not act as an agonist? The answer lies in a side chain. This side chain contains an amino group that is protonated and forms a hydrogen bond to

## BOX 8.2 Oestradiol and the oestrogen receptor *(Continued)*

Asp-351—an interaction that does not take place with oestradiol. In doing so, the side chain protrudes from the binding pocket and prevents the receptor helix H12 folding over as a lid. As a result, the AF-2 binding region is not exposed, the coactivator cannot bind and the transcription factor cannot be formed. Hence, the side chain is crucial to antagonism. It must contain an amine group of the correct basicity such that it ionizes and forms the interaction with Asp-351, and it must be of the correct length and flexibility to place the amine in the correct position for binding.

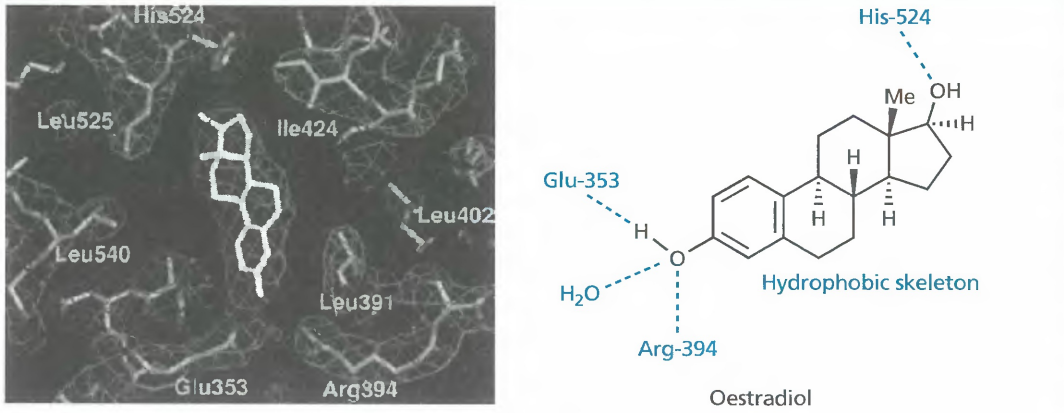



**FIGURE 1** Binding mode of oestradiol with the oestrogen receptor.

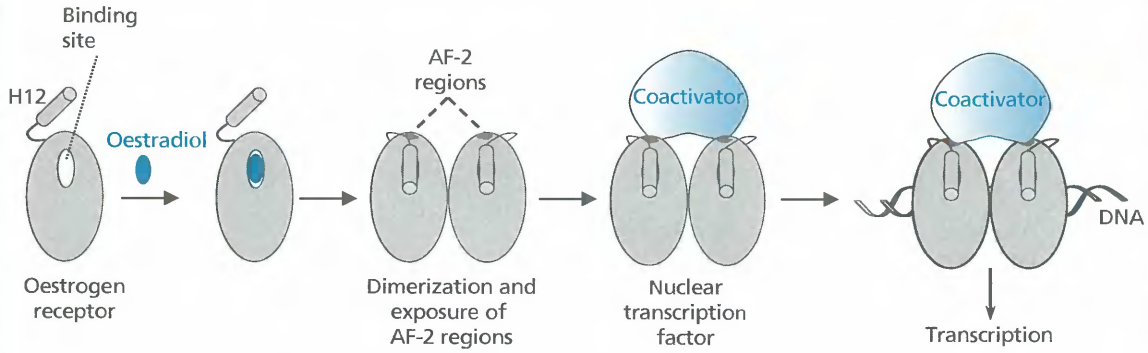



**FIGURE 2** Control of transcription by the oestrogen receptor.

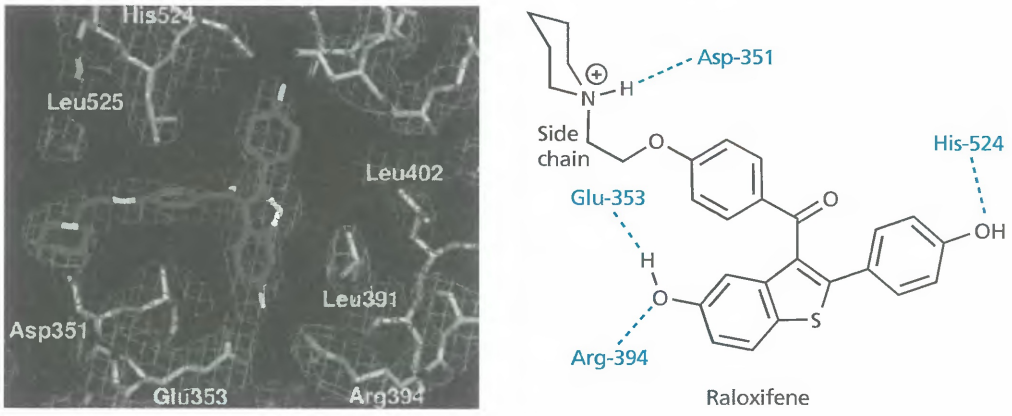



**FIGURE 3** Binding mode of raloxifene with the oestrogen receptor.

### 8.3.2 Antagonists acting outwith the binding site

There are examples of antagonists which do not bind to the binding site used by the natural chemical messenger. How do these antagonists work? There are two possible explanations.

**Allosteric modulators** Some receptors have allosteric binding sites. These are binding sites which are located on a different part of the receptor surface from the binding site, and which bind natural molecules called modulators that 'modulate' the activity of receptors by either enhancing it (section 8.2.6) or diminishing it. If activity is diminished, the modulator is acting indirectly as an antagonist. The mechanism by which this takes place could be viewed in a similar way to the allosteric inhibition of enzymes (section 3.6). The modulator binds to the allosteric binding site and causes it to change shape—an induced fit. This has a knock-on effect which alters the shape of the normal binding site. If the site becomes too distorted, then it is no longer able to bind the normal chemical messenger, or binds it less effectively. Therefore, it is possible to design an antagonist that will bind to the allosteric binding site rather than to the normal binding site (Fig. 8.13).

**Antagonism by the 'umbrella' effect** Some antagonists are thought to bind to regions of the receptor which are close to the normal binding site. Although they do not bind directly to the binding site, the molecule acts as a 'shield' or as an 'umbrella', preventing the normal messenger from accessing the binding site (Fig. 8.14).

## 8.4 Partial agonists

- A partial agonist obviously must bind to a receptor in order to have an agonist effect. However, it may be binding in such a way that the conformational change induced is not ideal, and the subsequent effects of receptor activation are decreased. For example, a receptor may be responsible for the opening of an ion channel. The normal chemical messenger causes an induced fit that results in the ion channel fully opening up. A partial agonist, on the other hand, binds to the receptor and causes a less significant induced fit, which results in only a slight distortion of the receptor. As a result, the ion channel is only partially opened (Fig. 8.15).
- The partial agonist may be capable of binding to a receptor in two different ways by using different binding regions in the binding site. One method of binding activates the receptor (an agonist effect), but the other does not (an antagonist effect). The balance of agonism versus antagonism would then depend on the relative proportions of molecules binding by either method.

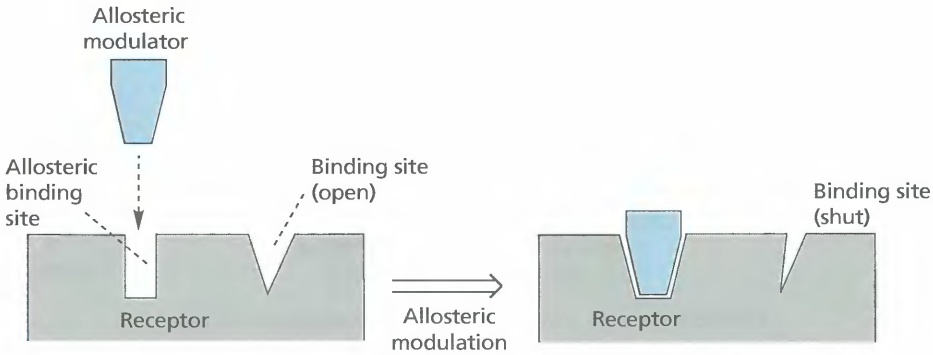



**FIGURE 8.13** Principle by which an allosteric antagonist distorts a binding site.

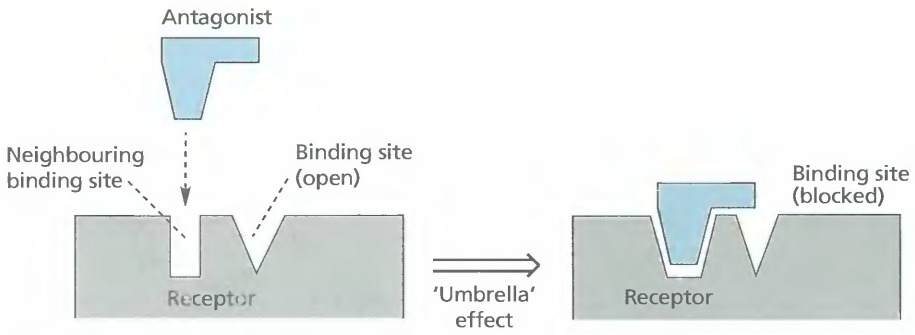



**FIGURE 8.14** Antagonism by the 'umbrella effect'.

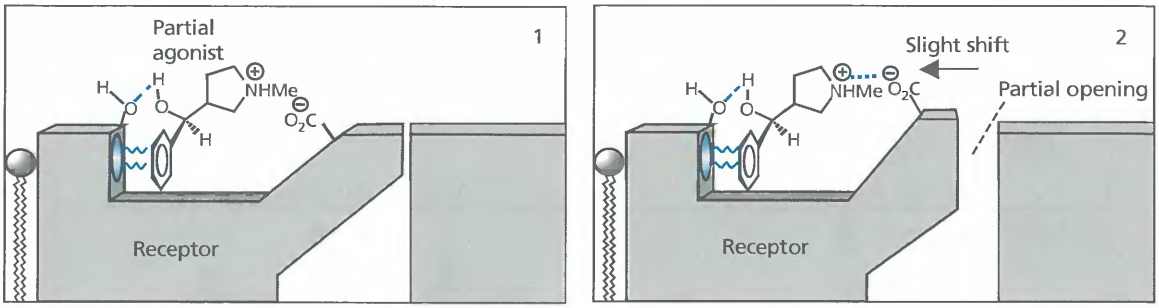



**FIGURE 8.15** Partial agonism.

This theory was used to explain the activity of partial agonists observed during the development of the anti-ulcer drug cimetidine (section 25.2.2.2).

- Receptors that bind the same chemical messenger are not all the same. The partial agonist may be capable of distinguishing between different receptor types or subtypes, acting as an agonist at one subtype, but as an antagonist at another subtype.

Examples of partial agonists in the opioid and anti-histamine fields are discussed in Chapters 24 and 25, respectively.

## 8.5 Inverse agonists

Many antagonists which bind to a receptor binding site are in fact more properly defined as inverse agonists. An inverse agonist has the same effect as an antagonist in that it binds to a receptor, fails to activate it, and prevents the normal chemical messenger from binding. However, there is more to an inverse agonist than that. Some receptors (e.g. the **GABA**[2], **serotonin** and **dihydropyridine receptors**) are found to have an inherent activity even in the absence of the chemical messenger. They are said to be constitutionally active. An inverse agonist is capable of preventing this activity as well.

The discovery that some receptors have an inherent activity has important implications for receptor theory. It suggests that these receptors do not have a 'fixed' inactive conformation, but are continually changing shape such that there is an equilibrium between the active conformation and different inactive conformations. In that equilibrium, most of the receptor population is in an inactive conformation but a small proportion of the receptors is in the active conformation. The action of agonists and antagonists is then explained by how that equilibrium is affected by binding preferences (Fig. 8.16).

If an agonist is introduced (frame B), it binds preferentially to the active conformation and stabilizes it, shifting the equilibrium to the active conformation and leading to an increase in the biological activity associated with the receptor.

In contrast, it is proposed that an antagonist binds equally well to all receptor conformations (both active and inactive) (frame C). There is no change in biological activity, as the equilibrium is unaffected. The introduction of an agonist has no effect either, because all the receptor binding sites are already occupied by the antagonist. Antagonists such as these will have some structural similarity to the natural agonist.

An inverse agonist is proposed to have a binding preference for an inactive conformation. This stabilizes the inactive conformation and shifts the equilibrium away from the active conformation leading to a drop in inherent biological activity (frame D). An inverse agonist need have no structural similarity to an agonist, as it could be binding to a different part of the receptor.

A partial agonist has a slight preference for the active conformation over any of the inactive conformations. The equilibrium is shifted to the active conformation but not to the same extent as with a full agonist, and so the increase in biological activity is less (frame E).

## 8.6 Desensitization and sensitization

Some drugs bind relatively strongly to a receptor, switch it on, but then subsequently block the receptor after a certain period of time. Thus, they are acting as agonists, then antagonists. The mechanism of how this takes place is not clear, but it is believed that prolonged binding of the agonist to the receptor results in phosphorylation of hydroxyl or phenolic groups in the receptor. This causes the receptor to alter shape to an inactive conformation, despite the binding site being occupied (Fig. 8.17). This altered tertiary structure is then maintained as long as the binding site is occupied by the agonist. When the drug eventually leaves, the receptor is dephosphorylated and returns to its original resting shape.

[2] GABA = γ-aminobutyric acid

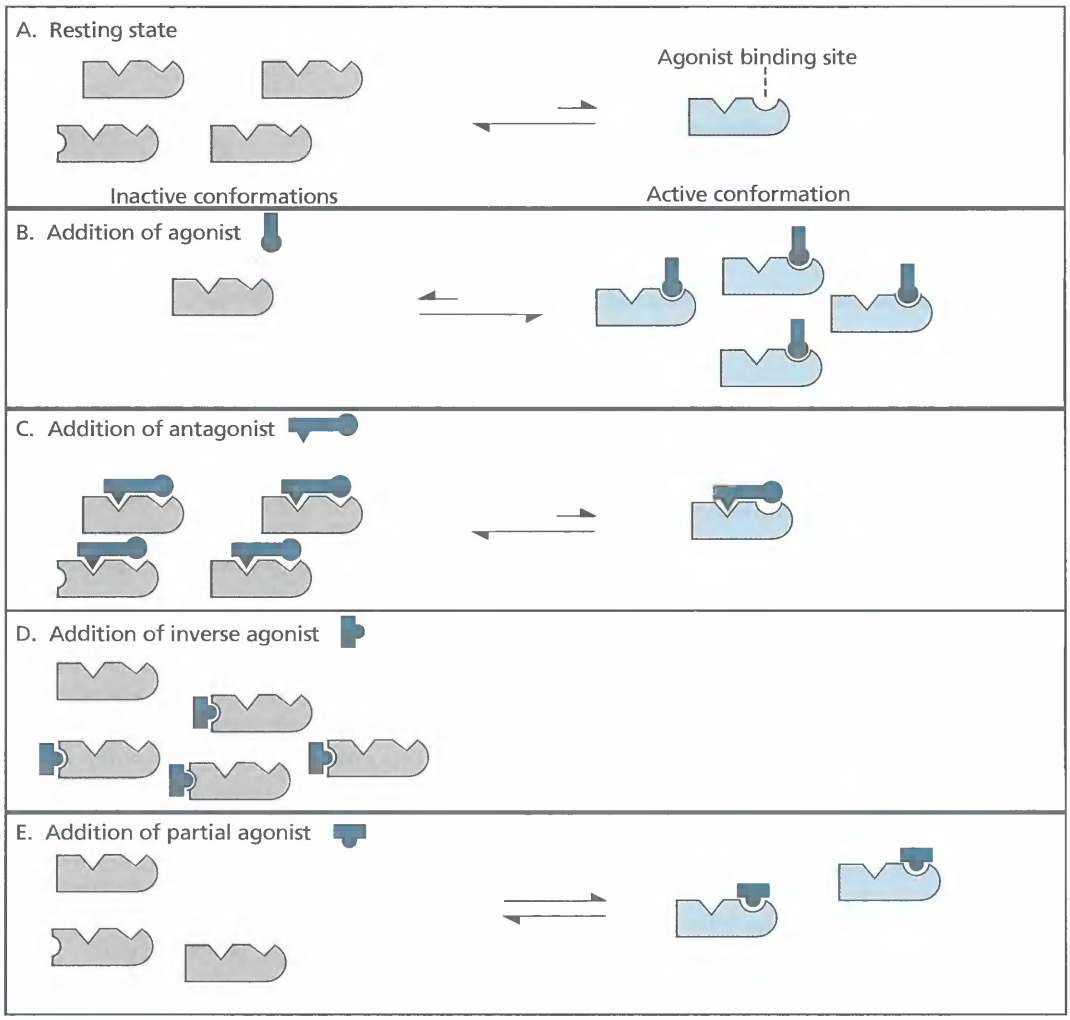



**FIGURE 8.16** Equilibria between active and inactive receptor conformations and the effect of agonists, antagonists, inverse agonists and partial agonists.

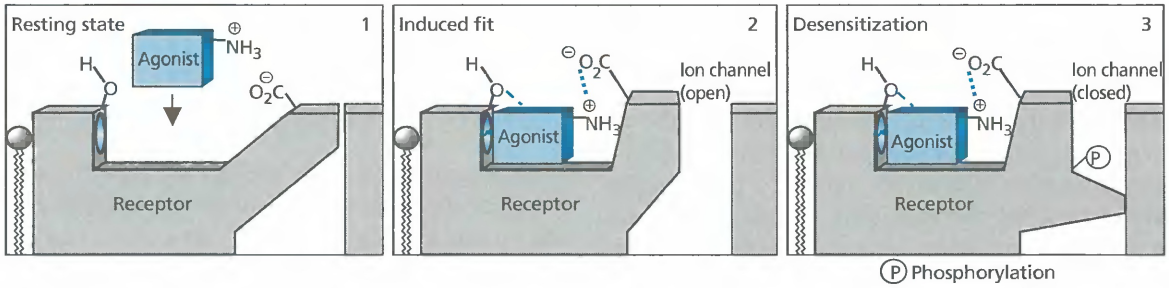



**FIGURE 8.17** Desensitization of a receptor following prolonged binding of an agonist.

On even longer exposure to a drug, the receptor–drug complex may be removed completely from the cell membrane by a process called **endocytosis**. Here, the relevant portion of the membrane is 'nipped out', absorbed into the cell, and metabolized.

Prolonged activation of a receptor can also result in the cell reducing its synthesis of the receptor protein. Consequently, it is generally true that the best agonists bind swiftly to the receptor, pass on their message, and then leave quickly. Antagonists, in contrast, tend to be slow to add and slow to leave. Prolonged exposure of a target receptor to an antagonist may lead to the opposite of desensitization (i.e. sensitization). This is where the cell synthesizes more receptors to compensate for

the receptors that are blocked. This is known to happen when some β-blockers are given over long periods (section 23.11.3).

## 8.7 Tolerance and dependence

As mentioned above, depriving a target receptor of its neurotransmitter may induce that cell to synthesize more receptors. By doing so, the cell gains a greater sensitivity for what little neurotransmitter is left. This process can explain the phenomena of tolerance and dependence (Fig. 8.18).

Tolerance is a situation where higher levels of a drug are required to get the same biological response. If a drug is acting to suppress the binding of a neurotransmitter, then the cell may respond by increasing the number of receptors. This would require increasing the dose to regain the same level of antagonism.

If the drug is suddenly stopped, then all the receptors suddenly become available. There is now an excess of receptors, which makes the cell supersensitive to normal levels of neurotransmitter. This would be equivalent to receiving an overdose of an agonist. The resulting biological effects would explain the distressing withdrawal symptoms resulting from stopping certain drugs. These withdrawal symptoms would continue until the number of receptors returned to their original level. During this period, the patient may be tempted to take the drug again in order to 'return to normal' and will have then acquired a dependence on the drug.

## 8.8 Receptor types and subtypes

The receptors for a particular chemical messenger are not all identical. There are various types and subtypes of receptor and it is found that different organs have a preference for some types or subtypes over others. This means that designing a drug which is selective for a particular type or subtype of receptor leads to selectivity of action against a particular organ in the body (see also sections 4.3 and 4.7.3).

Some examples of receptor types and subtypes are given in Table 8.1. The identification of many of these subtypes is relatively recent and the current emphasis in medicinal chemistry is to design drugs which are as selective as possible so that the drugs are tissue selective and have fewer side effects. For example, there are

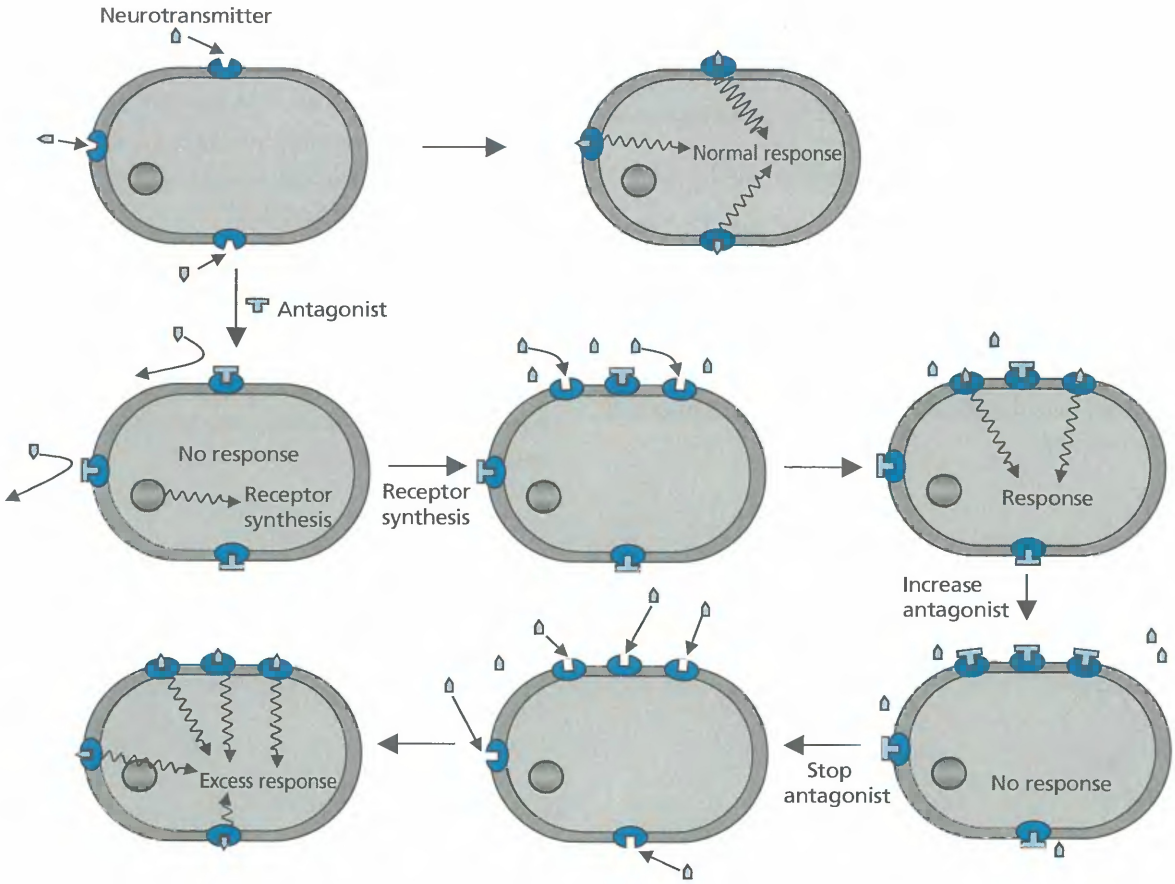



**FIGURE 8.18** Increasing cell sensitivity by the synthesis of more receptors.

**TABLE 8.1** Some examples of receptor types and subtypes

| Receptor | Type | Subtype | Examples of agonist therapies | Examples of antagonist therapies |
|---|---|---|---|---|
| Cholinergic | Nicotinic (N)<br>Muscarinic (M) | Nicotinic (4 subtypes) M1–M5 | Stimulation of GIT motility (M1)<br>Glaucoma (M) | Neuromuscular blockers and muscle relaxant (N)<br>Peptic ulcers (M1)<br>Motion sickness (M) |
| Adrenergic (adrenoceptors) | Alpha ($\alpha_1$, $\alpha_2$)<br>Beta ($\beta$) | $\alpha_{1A}$ $\alpha_{1B}$ $\alpha_{1D}$<br>$\alpha_{2A}$–$\alpha_{2C}$<br>($\beta_1$, $\beta_2$, $\beta_3$) | Anti-asthmatics ($\beta_2$) | $\beta$-blockers ($\beta_1$) |
| Dopamine | | $D_1$–$D_5$ | Parkinson's disease | Antidepressant ($D_2/D_3$) |
| Histamine | | $H_1$–$H_3$ | Vasodilation (limited use) | Treatment of allergies, anti-emetics, sedation ($H_1$)<br>Anti-ulcer agents ($H_2$) |
| Opioid and opioid-like | | $\mu$, $\kappa$, $\delta$, ORL1 | Analgesics ($\kappa$) | Antidote to morphine overdose |
| 5-Hydroxy-tryptamine (serotonin) | 5-$HT_1$–5-$HT_7$ | 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D-1F}$<br>5-$HT_{2A-2C}$<br>5-$HT_{5A}$<br>5-$HT_{5B}$ | Antimigraine (5-$HT_{1D}$)<br>Stimulation of GIT motility (5-$HT_4$) | Anti-emetics (5-$HT_3$)<br>Ketanserin 5-$HT_2$ |
| Oestrogen | | | Contraception | Breast cancer (Tamoxifen) |

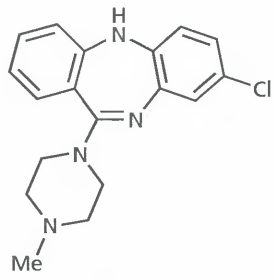
five types of dopaminergic receptor. All clinically effective antipsychotic agents (e.g. **clozapine**, **olanzapine**, and **risperidone**; Fig. 8.19) antagonize the dopaminergic receptors D2 and D3. However, blockade of D2 receptors may lead to some of the side effects observed, and a selective D3 antagonist may have better properties as an antipsychotic.

Other examples of current research projects include:

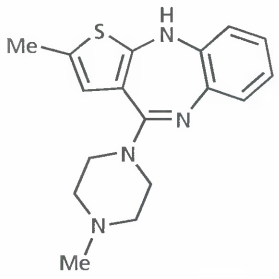
- muscarinic ($M_2$) agonists for the treatment of heart irregularities
- adrenergic ($\beta_3$) agonists for the treatment of obesity
- *N*-methyl-D-aspartate (NMDA) antagonists for the treatment of stroke
- cannabinoid ($CB_1$) antagonists for the treatment of memory loss.

**KEY POINTS**

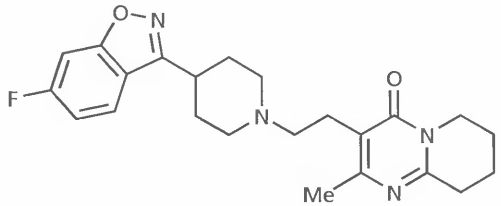
- Agonists are compounds that mimic the natural ligand for the receptor.
- Antagonists are agents that bind to the receptor, but which do not activate it. They block binding of the natural ligand.
- Agonists may have a similar structure to the natural ligand.
- Antagonists bind differently from the natural ligand such that the receptor is not activated.
- Antagonists can bind to regions of the receptor that are not involved in binding the natural ligand. In general, antagonists tend to have more binding interactions than agonists and bind more strongly.
- Partial agonists induce a weaker effect than a full agonist. Inverse agonists act as antagonists, but also eliminate any resting activity associated with a receptor.




Clozapine




Olanzapine




Risperidone

**FIGURE 8.19** Antipsychotic agents.

- Desensitization may occur when an agonist is bound to its receptor for a long period of time. Phosphorylation of the receptor results in a change of conformation.
- Sensitization can occur when an antagonist is bound to a receptor for a long period of time. The cell synthesizes more receptors to counter the antagonist effect.
- Tolerance is a situation where increased doses of a drug are required over time to achieve the same effect.
- Dependence is related to the body's ability to adapt to the presence of a drug. On stopping the drug, withdrawal symptoms occur as a result of abnormal levels of target receptor.
- There are several receptor types and subtypes, which vary in their distribution round the body. They also vary in their selectivity for agonists and antagonists.
- Pharmacodynamics is the study of how drugs interact with their targets. Pharmacokinetics is the study of how drugs reach their targets *in vivo*.

## 8.9 Affinity, efficacy, and potency

The affinity of a drug for a receptor is a measure of how strongly that drug binds to the receptor. Efficacy is a measure of the maximum biological effect that a drug can produce as a result of receptor binding. It is important to appreciate the distinction between affinity and efficacy. A compound with high affinity does not necessarily have high efficacy. For example, an antagonist can bind with high affinity but has no efficacy. The **potency** of a drug refers to the amount of drug required to achieve a defined biological effect—the smaller the dose required, the more potent the drug. It is possible for a drug to be potent (i.e. active in small doses) but have a low efficacy.

Affinity can be measured using a process known as **radioligand labelling**. A known antagonist (or ligand) for the target receptor is labelled with radioactivity and is added to cells or tissue such that it can bind to the receptors present. Once an equilibrium has been reached, the unbound ligands are removed by washing, filtration, or centrifugation. The extent of binding can then be measured by detecting the amount of radioactivity present in the cells or tissue, and the amount of radioactivity that was removed. The equilibrium constant for bound versus unbound radioligand is defined as the **dissociation binding constant** ($K_d$).

$$[L] + [R] \rightleftharpoons \underset{\text{Receptor-ligand complex}}{[LR]} \qquad K_d = \frac{[L] \times [R]}{[LR]}$$

[L] and [LR] can be found by measuring the radioactivity of unbound ligand and bound ligand respectively, after correction for any background radiation. However, it is not possible to measure [R], and so we have to carry out some mathematical manipulations to remove [R] from the equation.

The total number of receptors present must equal the number of receptors occupied by the ligand ([LR]) and those that are unoccupied ([R]), i.e.

$$R_{tot} = [R] + [LR].$$

This means that the number of receptors unoccupied by a ligand is:

$$[R] = R_{tot} - [LR].$$

Substituting this into the first equation and rearranging leads to the **Scatchard equation**, where both [LR] and [L] are measurable:

$$\frac{[\text{Bound ligand}]}{[\text{Free ligand}]} = \frac{[LR]}{[L]} = \frac{R_{tot} - [LR]}{K_d}$$

We are still faced with the problem that $K_d$ and $R_{tot}$ cannot be measured directly. However, these terms can be determined by drawing a graph based on a number of experiments where different concentrations of a known radioligand are used. [LR] and [L] are measured in each case and a **Scatchard plot** (Fig. 8.20) is drawn which compares the ratio [LR]/[L] versus [LR]. This gives a straight line, and the point where it meets the x-axis represents the total number of receptors available ($R_{tot}$) (line A; Fig. 8.21). The slope is a measure of the radioligand's affinity for the receptor and allows $K_d$ to be determined.

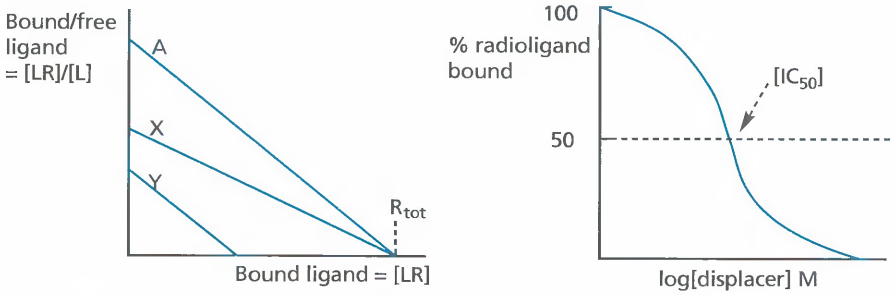



**FIGURE 8.20** Scatchard plot. A, radioligand only; X, radioligand + competitive ligand; Y, radioligand + non-competitive ligand.

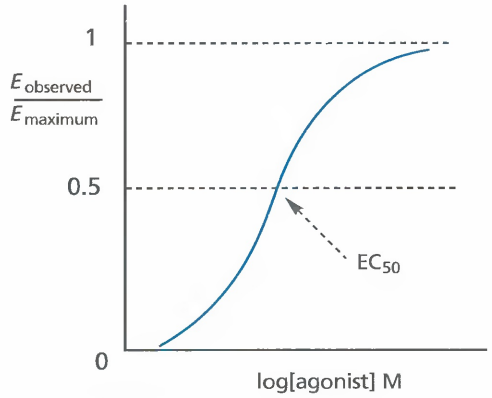



**FIGURE 8.21** Measurement of $EC_{50}$.

We are now in the position to determine the affinity of a novel drug that is not labelled. This is done by repeating the radioligand experiments in the presence of the unlabelled test compound. The test compound competes with the radioligand for the receptor's binding sites and is called a **displacer**. The stronger the affinity of the test compound, the more effectively it will compete for binding sites and the less radioactivity will be measured for [LR]. This will result in a different line in the Scatchard plot.

If the test compound competes directly with the radiolabelled ligand for the same binding site on the receptor, then the slope is decreased but the intercept on the x-axis remains the same (line X in the graph). In other words, if the radioligand concentration is much greater than the test compound it will bind to all the receptors available.

Agents that bind to the receptor at an allosteric binding site do not compete with the radioligand for the same binding site and so cannot be displaced by high levels of radioligand. However, by binding to an allosteric site they make the normal binding site unrecognizable to the radioligand and so there are less receptors available. This results in a line with an identical slope to line A, but crossing the x-axis at a different point, thus indicating a lower total number of available receptors (line Y).

The data from these displacement experiments can be used to plot a different graph which compares the percentage of the radioligand that is bound to a receptor versus the concentration of the test compound. This results in a sigmoidal curve termed the **displacement** or **inhibition curve**, which can be used to identify the **$IC_{50}$ value** for the test compound (i.e. the concentration of compound that prevents 50% of the radioactive ligand being bound).

The **inhibitory** or **affinity constant ($K_i$)** for the test compound is the same as the $[IC]_{50}$ value if non-competitive interactions are involved. For compounds that *are* in competition with the radioligand for the binding site, the inhibitory constant depends on the level of radioligand present and is defined as:

$$K_i = \frac{IC_{50}}{1 + [L]_{tot}/K_d}$$

where $K_d$ is the dissociation constant for the radioactive ligand and $[L]_{tot}$ is the concentration of radioactive ligand used in the experiment.

**Efficacy** is determined by measuring the maximum possible effect resulting from receptor-ligand binding. Potency can be determined by measuring the concentration of drug required to produce 50% of the maximum possible effect ($\mathbf{EC_{50}}$) (Fig. 8.21). The smaller the value of $EC_{50}$, the more potent the drug. In practice, $\mathbf{pD_2}$ is taken as the measure of potency where $pD_2 = -\log[EC]_{50}$.

A **Schild analysis** is used to determine the dissociation constant ($K_d$) of competitive antagonists (Fig. 8.22). An agonist is first used at different concentrations to activate the receptor, and an observable effect is measured at each concentration. The experiment is then repeated several times in the presence of different concentrations of antagonist. Comparing the effect ratio ($E_{observed}/E_{maximum}$) versus the log of the agonist concentration (log[agonist]) produces a series of sigmoidal curves

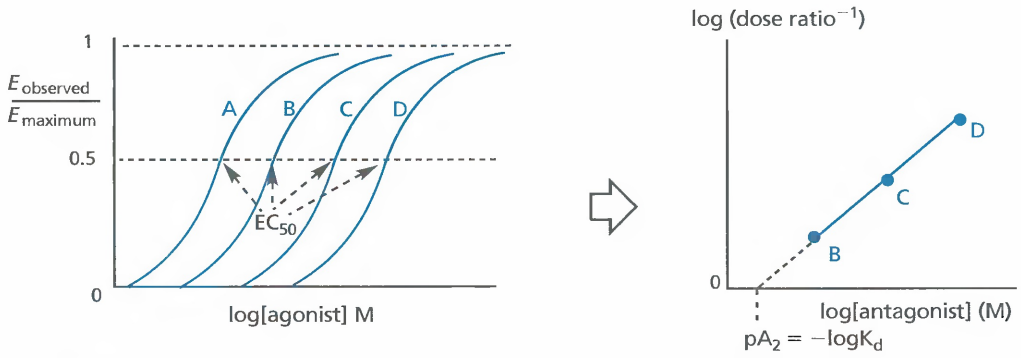



**FIGURE 8.22** Schild analysis. A, no antagonist present; B–D, increasing concentrations of antagonist present.

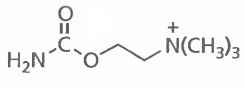
where the $EC_{50}$ of the agonist increases with increasing antagonist concentration. In other words, greater concentrations of agonist are required to compete with the antagonist. A **Schild plot** is then constructed, which compares the reciprocal of the dose ratio versus the log of the antagonist concentration. (The **dose ratio** is the agonist concentration required to produce a specified level of effect when no antagonist is present, compared to the agonist concentration required to produce the same level in the presence of antagonist.) The line produced from these studies can be extended to the x-axis to find $pA_2$ ($= -\log K_d$), which represents the affinity of the competitive antagonist.

**KEY POINTS**

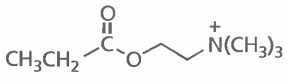
- Affinity is a measure of how strongly a drug binds to a receptor. Efficacy is a measure of the effect of that binding on the cell. Potency relates to how effective a drug is in producing a cellular effect.
- Affinity can be measured from Scatchard plots derived from radioligand displacement experiments.
- Efficacy is determined by the $EC_{50}$ value—the concentration of agent required to produce 50% of the maximum possible effect resulting from receptor activation.
- A Schild analysis is used to determine the dissociation constant of competitive antagonists.

## QUESTIONS

**1.** Structure I is an agonist which binds to the cholinergic receptor and mimics the action of the natural ligand acetylcholine. Structure II on the other hand shows no activity and does not bind to the receptor. Suggest why this might be the case.




(I)

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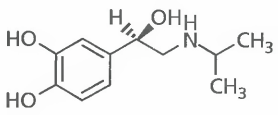
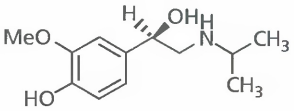
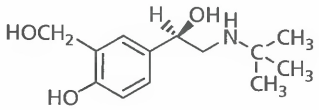



Isoprenaline




Inactive metabolite




*R*-Salbutamol

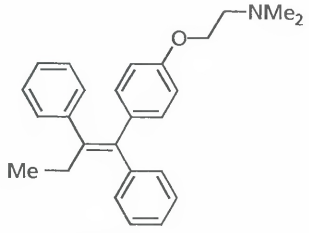
**2.** Isoprenaline undergoes metabolism to give the inactive metabolite shown above. Suggest why this metabolite is inactive.

**3.** Salbutamol is an anti-asthmatic agent, which acts as an adrenergic agonist. Do you think it is likely to show any selectivity between the α- or β-adrenoceptors? Explain your answer.

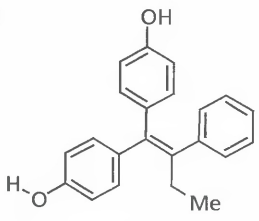
**4.** Propranolol (Fig. 23.25) is an adrenergic antagonist. Compare the structure of propranolol with noradrenaline and identify which features are similar in both molecules. Suggest why this molecule might act as an antagonist rather than an agonist and whether it might show any selectivity between the different types of adrenergic receptor.

**5.** If you were asked to design drugs that acted as selective antagonists of the dopamine receptor, what structures might you consider synthesizing?

**6.** Tamoxifen acts as an antagonist for the oestrogen receptor. Suggest how it might bind to the receptor in order to do this.




Tamoxifen




Tamoxifen metabolite

**7.** The tamoxifen metabolite shown acts as an oestrogen agonist rather than an antagonist. Why?

## FURTHER READING

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*Titles for general further reading are listed on p. 725.*