Drugs used in medicine may alter the metabolism of cells and organs in the human body, or they may kill or inactivate pathogens such as bacteria, viruses and fungi. Regardless of their effect, all drugs work by binding to specific target molecules within the organism. These target sites can be of two different types, enzymes or receptor sites.

In this unit you will look at how drugs bind to these target sites and start to understand how this affects the metabolism.

On successful completion of this topic you will:
• understand the role of enzymes and receptors as drug targets (LO1).

To achieve a Pass in this unit you need to show that you can:
• explain the role of enzymes and receptors as drug target sites (1.1)
• explain drug-receptor binding interactions (1.2)
• distinguish between competitive and non-competitive enzyme inhibition (1.3)
• explain the relationship between receptors and drug affinity, efficacy and potency (1.4).
1 Enzymes as drug targets

Enzymes

Enzymes are macromolecules, usually proteins, which act as biological catalysts – they create a new pathway that allows a reaction to occur many times faster than the uncatalysed reaction.

Drug case study

The action of many antibiotics is a result of the antibiotic drug targeting specific enzymes in the metabolism of bacteria. For example, penicillin (discussed in detail in Topic guide 9.4, section 1) acts by binding to an enzyme in bacteria that plays a key role in the construction of the bacterial cell wall.

Mechanism of enzyme action

Knowledge of the way in which enzymes work is important in the design of drugs that target enzymes.

Enzymes act on specific molecules called substrates that bind to a region of the enzyme known as the active site (or binding site) to form an enzyme substrate complex, e.g. as shown in Figure 9.1.1. Only specific substrates can fit and bind to the active site because the substrate and active site have complementary shapes and charges; drug molecules are often designed to mimic the shape of the substrate molecule.

Non-competitive inhibitor

Non-competitive binding site

Figure 9.1.1: Structure of an enzyme showing the active site, substrate and non-competitive binding site.

Inhibitors

The ability of drugs to affect enzyme activity, and hence alter metabolism in cells, is due to their ability to act as inhibitor molecules.

Inhibitors can be classified as non-reversible or reversible.

- Non-reversible inhibitors: these bind strongly to the active site but are not converted into product molecules.
- Reversible inhibitors, which are of two types:
  - competitive inhibitors – these have similar structures to substrate molecules and can bind to the active site in a similar way. The inhibitor and the substrate are competing for the active site and hence inhibition can be reversed by increasing substrate concentration.
  - non-competitive inhibitors – these bind to a second binding site (often called an allosteric site). The binding decreases enzyme activity by producing a conformational change in the enzyme structure. Inhibition can be reversed only by reducing the inhibitor concentration.
Some inhibitors act by a mixed mechanism, bonding to both the active and allosteric site. **Table 9.1.1** shows some examples of drugs that operate as inhibitors.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
<th>Type of inhibition</th>
<th>Description of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Analgesic (pain relief)</td>
<td>Non-reversible</td>
<td>Prevents the binding of arachidonic acid to a cyclooxygenase (COX) enzyme, necessary for the formation of prostaglandins</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Cancer treatment</td>
<td>Competitive</td>
<td>Competes with tetrahydrofolate for the active site of dihydrofolate reductase, necessary for nucleotide synthesis</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Anti-bacterial action on oral bacteria (added to toothpaste and some water supplies)</td>
<td>Non-competitive</td>
<td>Binds to a site on the enolase enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate during glycolysis</td>
</tr>
</tbody>
</table>

**Activity**

Use research to find out more about the effect of each of the drugs in **Table 9.1.1**. You should be able to explain how the described effect shown above might link with the way in which these drugs are used (for example, why preventing nucleotide synthesis would be useful in a drug designed to treat cancer).

**Other receptor sites**

You will find out more about the way in which drugs interact with other targets, such as receptor sites on the surface of cells and ion-channels, on pages 9–12 of this topic guide.

**Checklist**

You should now be familiar with the following ideas about drugs and enzymes:

- most drugs work by binding to specific target sites
- the bonding of drugs to target sites produces changes in the metabolism of human cells or in pathogenic organisms
- enzymes are important examples of substances with active target sites
- an enzyme has an active site that enables it to bind to a substrate molecule
- an enzyme can be inhibited by a molecule or ion that binds to the active site or another site on the enzyme
- types of inhibition include irreversible, reversible competitive and reversible non-competitive.

**2 How do drugs bind to receptor sites?**

Enzymes and most other receptor sites are proteins. They contain polypeptide chains that consist of amino acid residues bonded together by peptide links.

There are 20 different amino acids found in protein molecules; they differ in the nature of the side group and these side groups are responsible for the interactions that bind the drug molecule to the receptor site.
Types of drug-receptor binding interactions

Drug-receptor binding makes use of a range of types of chemical bonding: ionic, hydrogen-bonding, Van der Waals interactions and covalent bonding. Drug molecules that bond to receptors in this way are often described as ligands. Various types of protein-ligand bonding are shown in Figure 9.1.2.

The folding of the protein chain that creates the shape of the active site allows specific side groups to come into close alignment with groups in the substrate and form bonds (see Figure 9.1.3 for an example).

Functional groups involved in drug-receptor sites

Take it further

Table 9.1.2, on the following page, shows the names of amino acids as three letter abbreviations. The full names and structures of the 20 naturally occurring amino acids can be found in standard biochemistry textbooks or online at sites such as: www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/aastruct.html.
### Table 9.1.2: The functional groups present in some amino acids.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Amino acid residue containing this group</th>
<th>Types of interactions involving this group</th>
</tr>
</thead>
</table>
| \(-\text{COOH}\) (becomes \(\text{COO}^-\) in alkaline conditions) | asp, glu, | Ionic (in ionised form)  
Hydrogen bonding (in unionised form) |
| \(-\text{NH}_2\) or \(-\text{NH}^-\) (becomes \(-\text{NH}_3^+\) or \(-\text{NH}_2^+\) in acidic conditions) | lys, arg, his | Ionic (in ionised form)  
Hydrogen bonding (in un-ionised form) |
| \(-\text{OH}\) | ser, tyr, thr | Hydrogen bonding |
| \(-\text{CH}_3\) (and longer hydrocarbon chains) | ala, val, leu, ile, met | Van der Waals |
| \(-\text{C}_6\text{H}_5\) | phe, tyr, trp | Van der Waals |

### Checklist
You should now be familiar with the following ideas about drug-receptor interactions:
- most receptor sites are proteins
- a range of electrostatic forces exist which permit binding between the drug and the receptor site
- the binding involves a range of amino acid side groups on the surface of the receptor site.

### Portfolio activity (1.2)
Use a suitable source of information to find the molecular structure of a drug molecule. Look at the functional groups in the drug molecule and suggest possible interactions that may form between these functional groups and a protein-based receptor site. A good example to use might be captopril, used to treat high blood pressure (see Topic 9.4 for more details). The structure of this molecule and that of the receptor site should be easily found by searching on the internet.

### 3 Enzyme inhibition and drug action

#### Making use of different types of inhibition

You read on pages 2–3 that enzymes are the target of many drug molecules, and that these drugs work by inhibiting enzymes.

#### Case study: Using different types of inhibition

During the identification and development of new drugs it will be important to establish what type of inhibition is involved, as drugs operating by different mechanisms of inhibition may be used in different ways. For example:
- **irreversible inhibition** (e.g. in the action of aspirin) permanently deactivates an enzyme, often at low inhibitor concentration. This could be helpful in drugs designed to target pathogenic microorganisms or for treatment of chronic pain.
- **reversible competitive inhibition** (e.g. in the action of methotrexate) is reversed when the ratio of substrate concentration to inhibitor concentration rises. This may mean that the drug may only be effective in high concentrations, increasing the possibility of harmful side effects.
- **reversible non-competitive inhibition** (e.g. in the action of fluoride) occurs at a separate binding site, not the active site, and hence the drug molecule may have no structural similarity to the substrate of the enzyme. This may increase the stability of the drug as there may be no natural pathways for their degradation. As a result the drug may need to be administered less frequently or at a lower concentration.
Distinguishing between different types of reversible inhibition

As you saw in the case study on page 5, the type of inhibition involved in the mechanism of drug action has some important implications for how it can be used.

This can easily be done in the laboratory by carrying out kinetic studies to find how the rate (or velocity) of an enzyme-catalysed reaction depends on the concentrations of substrate and inhibitor.

Michaelis-Menten plots

Experimenters can carry out investigations to measure the rate (reaction velocity, $V$) of an enzyme-catalysed reaction at different concentrations.

A graph of reaction velocity against substrate concentration is known as a Michaelis-Menten plot. The graph tends asymptotically towards $V_{\text{max}}$ because at high substrate concentrations the enzyme is becoming fully saturated with substrate. When this happens, the enzyme is working at maximum rate. $V_{\text{max}}$ is often expressed as the turnover rate (number of substrate molecules converted into product molecules per second).

Finding $V_{\text{max}}$ enables experimenters to find the value of $K_m$ (the Michaelis constant). $K_m$ can be calculated by finding the substrate concentration at which the reaction velocity is one-half of the maximum ($V_{\text{max}}/2$).

The value of this constant for an enzyme is a useful measure of how well the enzyme binds the substrate.

Applying the Michaelis-Menten model to enzyme inhibition

Laboratory investigations can be used to distinguish between the two main types of reversible inhibition.

Reaction rate is measured at different substrate concentrations and repeated with a range of different inhibitor concentrations.
The graphs in Figure 9.1.5 show several important differences:

- Both graphs show that reaction rate is reduced in the presence of inhibitors. However, in the case of competitive inhibition, the inhibition can be overcome if substrate concentration is high enough.
- For competitive inhibition, $V_{\text{max}}$ remains unaltered in the presence of an inhibitor; for non-competitive inhibition $V_{\text{max}}$ decreases to a new value, depending on the inhibitor concentration.
- For competitive inhibition, $K_m$ increases in the presence of an inhibitor; for non-competitive inhibition $K_m$ remains unaltered.

### Lineweaver-Burk plots

Accurately measuring $K_m$ and $V_{\text{max}}$ from Michaelis-Menten plots, and hence distinguishing competitive from non-competitive inhibition, was once very difficult because the graph needed to be extrapolated by hand. Graph-plotting software now avoids this problem, but a second method exists, which can be used as an alternative.

In this method, called the Lineweaver-Burk plot (or double reciprocal plot), the reciprocal of the reaction velocity, $1/V$, is plotted against the reciprocal of substrate concentration $1/[S]$.

This yields a straight line, given by the following equation:

$$
\frac{1}{V_0} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}} [S]}
$$

Comparing with the general equation for a straight line,

$$
y = c + mx,
$$

this suggests:

- the slope of the graph $= \frac{K_m}{V_{\text{max}}}$
- the y intercept $= \frac{1}{V_{\text{max}}}$
- the x-intercept $= -\frac{1}{K_m}$

Figure 9.1.6 shows an example of a Lineweaver-Burk plot.
Case study

New drug molecules are continually being developed to act as competitive inhibitors of metabolic enzymes. During the screening process to assess the possible use of a molecule as a drug, its ability to bind to the active site will be assessed. Michaelis-Menten or Lineweaver-Burk plots can be used to provide useful information about this. The $K_m$ value with and without an inhibitor present can be measured and from these values the dissociation constant of the enzyme-inhibitor complex, $K_i$ as well as the $IC_{50}$ value of the inhibitor. Both of these are important measures of the binding of the inhibitor.

1. What happens to the $K_m$ value of an enzyme in the presence of a competitive inhibitor?
2. If the $IC_{50}$ value of an inhibitor is small, what does this tell you about the strength of the bond between the inhibitor and the enzyme?

Lineweaver-Burk plots for competitive and non-competitive inhibitors

These are shown in Figure 9.1.7, and demonstrate what you already know from the previous section – that $K_m$ and $V_{max}$ are affected in different ways in these two modes of inhibition. Table 9.1.3 gives a summary of the effects.

![Figure 9.1.7: Non-competitive and competitive inhibitors show different patterns of kinetics in Lineweaver-Burk plots.](image)

<table>
<thead>
<tr>
<th>Type of inhibition</th>
<th>Effect on x-intercept ($-1/K_m$)</th>
<th>Effect on y-intercept ($1/V_{max}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>Decreases</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Non-competitive</td>
<td>Unchanged</td>
<td>Increases</td>
</tr>
</tbody>
</table>

Portfolio activity (1.3)

You can generate evidence for your portfolio by carrying out practical work on enzyme kinetics.

1. Select a reaction to study. It should be one where a measure of the rate of the reaction can be obtained using the equipment available to you, and where there are known inhibitor(s).
2. Carry out suitable experiments generating data for reaction rates. Ensure that you have the correct range of data to allow you to construct suitable plots. Alternatively, secondary data can be obtained from websites or textbooks.
3. Describe what is meant by competitive and non-competitive inhibition.
4. Plot suitable graphs that will enable you to identify what type of inhibition is operating in the reaction.
5. Explain how your graphs enable you to identify the type of inhibition.

Activity

Use ideas about how $V_{max}$ and $K_m$ change in the presence of different types of inhibitor to explain the pattern in the way the intercepts change in the Lineweaver-Burk plots.
9.1: Drugs and receptor sites

4 Receptors and drug action

In the previous section you read about how enzymes are used as targets for drugs; the physiological effect is caused by the inhibition of these enzymes.

In this section you will find out more about the other main receptor types used as drug targets and some of the implications for health professionals involved in the prescribing and administration of these drugs.

Types of receptors

There are four main types of receptors that act as drug targets:

- ion channels, which can open or close in response to the drug binding
- enzyme-coupled receptors – receptors on the outer surface of cell membranes which can activate enzymes on the interior surface of the membrane
- G protein-coupled receptors – receptors on the outer surface of cell membranes that can activate G proteins on the interior surface of the membrane. These in turn activate enzymes, which produce chemical messengers such as cyclic AMP
- receptors within the cell, for example sites in the nucleus which control transcription.

These receptor molecules all have a specific role in the control of cellular processes and there are naturally occurring ligands that bind to the receptor molecule, forming a ligand-receptor complex. Ligands usually have a complementary structure to the receptor site, so this is a very similar situation to the enzyme-substrate mechanism discussed in the previous section.

Many drug molecules act as ligands for a range of receptor sites, which explains the wide range of physiological effects produced by some drugs.
Signal transduction

Two of these types of receptor involve signal transduction. In these cases the effect of a drug on an enzyme is not a direct one. The drug molecule may bind to a receptor site (the recognition site) outside the cell – if that receptor site is linked in some way to an enzyme system (the effector) within that cell, then a new, specific response may be created within it. Figure 9.1.8 shows this in diagrammatic form.

Take it further

In How Drugs Work (McGavock, 2010), p36, the transduction process is described using the analogy of a doorbell; the bell button is the receptor receiving the external signal, which is then transduced into an entirely different signal by the effector (the chiming of the bell) inside the house. This helpful image is typical of the accessible writing in this book, which gives excellent, clear coverage of the action of drugs.

Activity

Use suitable references to research the transduction pathway involving the G protein, adenylate cyclase and cyclic AMP. Find some examples of drugs that affect this pathway and, for one of these drugs, briefly describe how it does this.

Agonists and antagonists

If a drug produces the same response as that of the natural ligand when it binds to a receptor site, it is known as an agonist.

In other cases, a drug molecule may bind to the receptor site without causing a response. If, by binding in this way, the drug prevents further ligands binding (by blocking the site or causing a conformational change) then the drug molecule is known as an antagonist.

Table 9.1.4 shows an example of each type of drug.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Used to treat</th>
<th>Mechanism of action</th>
<th>Agonist or antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>Angina, hypertension</td>
<td>Binds to ion channel protein on surface of smooth muscle cells, preventing calcium ions from crossing membrane and altering electrical potential of cell</td>
<td>antagonist</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>Asthma</td>
<td>Binds to recognition site on surface of membrane. This activates the G protein on the internal surface, stimulating cAMP production (see Activity above)</td>
<td>agonist</td>
</tr>
</tbody>
</table>

Affinity and efficacy

When a medical professional is taking decisions about suitable dose levels of a drug there are several very important aspects of drug biochemistry to take into account.

Some of these are related to the way in which drugs are absorbed and broken down by the body.
One key factor is related to how the effect of the drug varies with drug concentration. This is not a linear relationship, as shown in Figure 9.1.9. Notice in this diagram how a logarithmic scale is used for the x-axis.

**Explaining patterns of drug effect**

The effect of both of these drugs increases as the concentration of the drug increases because more drug-receptor complexes are formed. However, agonist drugs compete with the natural ligand for the receptor site so the percentage of binding sites occupied by drug molecules will always be slightly less than 100% even at very high drug concentrations. This explains why the graph levels off to a maximum. The concentration at which this happens depends on the relative affinity of the receptor for the drug and the natural ligand.

In the case of Drug B, the maximum effect is never approached. This is because the drug produces a lower level of response than Drug A – it is described as having a lower efficacy.

The potency of the drug is therefore affected by both affinity and efficacy. Potency is often measured by calculating the EC50 concentration (the concentration of a drug needed to produce an effect equal to 50% of the maximum effect). For an inhibitor molecule, this figure is known as the IC50 value and represents the concentration needed to inhibit a biological process to 50% of its level in the absence of an inhibitor (see the Case study for a real-life example).

**Activity**

Use the graph showing the effects of Drug A and Drug B in Figure 9.1.9 to calculate a value for the potency (EC50 value) of each drug.

**Case study**

During the screening process to assess the possible use of drugs as inhibitors of enzymes, a value of the IC50 is often calculated, either by biological assays or by computer modelling. The lower the IC50 value, the more likely the molecule is to be able to be used as a drug.

In Topic guide 9.3, section 1 you will see how gradually decreasing values of IC50 for BACE-1 inhibitors were used to show how the lead compound had become optimised.

**Tolerance and dependency**

**Tolerance**

Taking high doses of drugs over an extended period of time can lead to tolerance. This means that the concentration required to produce a given level of effect will progressively increase.
Tolerance can be psychological, but there are also physiological mechanisms that can produce it. For example, continued use of opiates such as morphine (prescribed for relief of extreme pain) can create tolerance, possibly due to molecules involved in signal transduction permanently altering the conformation of receptor sites.

**Take it further**

More details about the research into opiate tolerance can be found at [http://www.opioids.com/morphine/dependence.html](http://www.opioids.com/morphine/dependence.html)

Tolerance may also be linked to an increase in the rate at which the drug is degraded in the body.

I am a nurse in a hospice. We provide care for people in final stages of terminal illnesses, such as cancer. The philosophy of the hospice movement is to provide an environment that maintains the dignity of an individual while providing pain relief to make their final days as comfortable as possible. Many patients will be able to self-medicate with morphine using oral suspensions and this allows them to have a sense of control over their medication regimen, balancing pain relief against their desire to stay as alert and awake as possible.

One thing that we must be aware of in use of morphine is the possibility of rapidly building up a physiological tolerance, meaning that higher and higher doses are needed to achieve the same level of pain relief. These higher concentrations that the patient may need to use could create other problems such as the possible suppression of respiration. We are looking into the possibility of prescribing additional drugs that may be able to slow the development of this tolerance. Other issues, such as that of dependence, are of secondary importance in this end-of-life care as the timescale for the use of morphine is, by its very nature, limited.

**Dependency**

In the case of opiate-based drugs, the development of tolerance often goes hand in hand with that of dependence. Drugs such as opiates stimulate dopamine production that, in turn, acts on the ‘pleasure’ centre of the brain, creating a psychological craving for more when levels drop.

**Portfolio activity (1.4)**

Use research to find information about a drug which binds to a specific type of receptor. Good examples could include captopril, which you encountered in an earlier activity, and dopamine.

1. Identify the type of receptor to which it binds.
2. Explain whether it is an agonist or antagonist.
3. Describe what is meant by the terms affinity, efficacy and potency and comment on any data you can find relating to these factors.
4. Explain how tolerance and dependence arise and comment on their significance (if any) in the use of your chosen drug.
Checklist
At the end of this section, you should be familiar with the following ideas:
✓ there are four main types of receptors
✓ drugs act as ligands, binding to these receptors
✓ drugs can act as agonists or antagonists
✓ the potency of a drug depends on its affinity for the receptor and its efficacy
✓ the development of tolerance and dependence are other issues which need to be considered by practitioners.

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