Section I Basic Principles

Chapter 1

Drug Passage across the Cell Membrane

Many drugs need to pass through one or more cell membranes to reach their site of action. A common feature of all cell membranes is a phospholipid bilayer, about 10 nm thick, arranged with the hydrophilic heads on the outside and the lipophilic chains facing inwards. This gives a sandwich effect, with two hydrophilic layers surrounding the central hydrophobic one. Spanning this bilayer or attached to the outer or inner leaflets are glycoproteins, which may act as ion channels, receptors, intermediate messengers (G-proteins) or enzymes. The cell membrane has been described as a 'fluid mosaic' as the positions of individual phosphoglycerides and glycoproteins are by no means fixed (Figure 1.1). An exception to this is a specialised membrane area such as the neuromuscular junction, where the array of postsynaptic receptors is found opposite a motor nerve ending.

The general cell membrane structure is modified in certain tissues to allow more specialised functions. Capillary endothelial cells have fenestrae, which are regions of the endothelial cell where the outer and inner membranes are fused together, with no intervening cytosol. These make the endothelium of the capillary relatively permeable; fluid in particular can pass rapidly through the cell by this route. In the case of the renal glomerular endothelium, gaps or clefts exist between cells to allow the passage of larger molecules as part of filtration. Tight junctions exist between endothelial cells of brain blood vessels, forming the blood–brain barrier, intestinal mucosa and renal tubules. These limit the passage of polar molecules and also prevent the lateral movement of glycoproteins within the cell membrane, which may help to keep specialised glycoproteins at their site of action (e.g. transport glycoproteins on the luminal surface of intestinal mucosa) (Figure 1.2).

Methods of Crossing the Cell Membrane

Passive Diffusion

This is the commonest method for crossing the cell membrane. Drug molecules move down a concentration gradient, from an area of high concentration to one of low concentration, and the process requires no energy to proceed. Many drugs are weak acids or weak bases and can exist in either the unionised or ionised form, depending on the pH. The unionised form of a drug is lipid-soluble and diffuses easily by dissolution in the lipid bilayer. Thus the rate at which transfer occurs depends on the pK_a of the drug in question. Factors influencing the rate of diffusion are discussed below.

In addition, there are specialised **ion channels** in the membrane that allow intermittent passive movement of selected ions down a concentration gradient. When opened, ion channels allow rapid ion flux for a short time (a few milliseconds) down relatively large

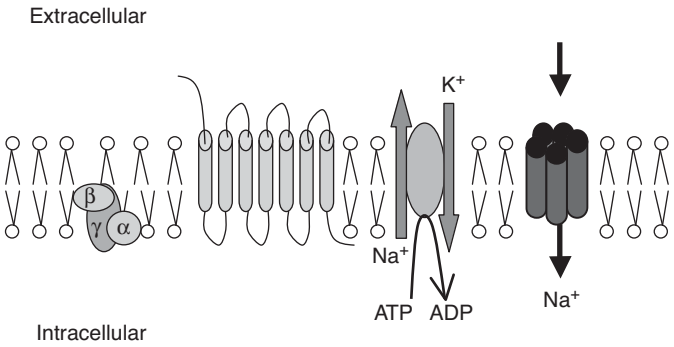

Figure 1.1 Representation of the cell membrane structure. The integral proteins embedded in this phospholipid bilayer are G-protein, G-protein-coupled receptors, transport proteins and ligand-gated ion channels. Additionally, enzymes or voltage-gated ion channels may also be present.

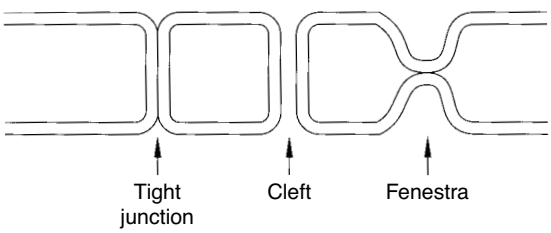

Figure 1.2 Modifications of the general cell membrane structure.

concentration and electrical gradients, which makes them suitable to propagate either ligand- or voltage-gated action potentials in nerve and muscle membranes.

The acetylcholine (ACh) receptor has five subunits (pentameric) arranged to form a central ion channel that spans the membrane (Figure 1.3). Of the five subunits, two (the α subunits) are identical. The receptor requires the binding of two ACh molecules to open the ion channel, allowing ions to pass at about 10^7 s^{-1}. If a threshold flux is achieved, depolarisation occurs, which is responsible for impulse transmission. The ACh receptor demonstrates selectivity for small cations, but it is by no means specific for Na^+. The $GABA_A$ receptor is also a pentameric, ligand-gated channel, but selective for anions, especially the chloride anion. The NMDA (N-methyl D-aspartate) receptor belongs to a different family of ion channels and is a dimer; it favours calcium as the cation mediating membrane depolarisation.

Ion channels may have their permeability altered by endogenous compounds or by drugs. Local anaesthetics bind to the internal surface of the fast Na^+ ion channel and prevent the conformational change required for activation, while non-depolarising muscle relaxants prevent receptor activation by competitively inhibiting the binding of ACh to its receptor site.

Facilitated Diffusion

Facilitated diffusion refers to the process where molecules combine with membrane-bound carrier proteins to cross the membrane. The rate of diffusion of the molecule–protein complex is still down a concentration gradient but is faster than would be expected by

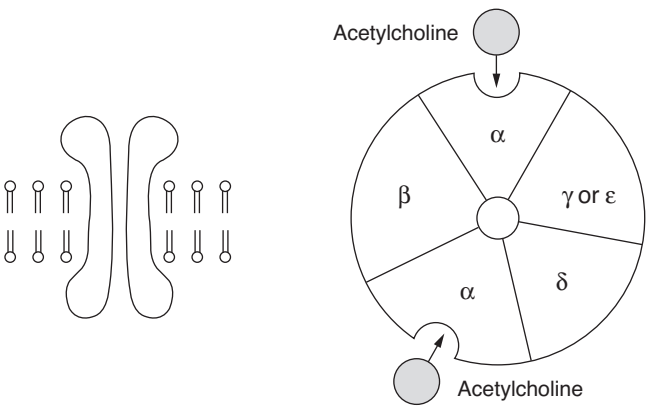

Figure 1.3 The acetylcholine (ACh) receptor has five subunits and spans the cell membrane. ACh binds to the α subunits, causing a conformational change and allowing the passage of small cations through its central ion channel. The ε subunit replaces the fetal-type γ subunit after birth once the neuromuscular junction reaches maturity.

diffusion alone. An example of this process is the absorption of glucose, a highly polar molecule, which would be relatively slow if it occurred by diffusion alone. There are several transport proteins responsible for facilitated glucose diffusion; they belong to the solute carrier (SLC) family 2. The SLC proteins belonging to family 6 are responsible for transport of neurotransmitters across the synaptic membrane. These are specific for different neurotransmitters: SLC6A3 for dopamine, SLC6A4 for serotonin and SLC6A5 for noradrenaline. They are the targets for certain antidepressants; serotonin-selective re-uptake inhibitors (SSRIs) inhibit SLC6A4.

Active Transport

Active transport is an energy-requiring process. The molecule is transported against its concentration gradient by a molecular pump, which requires energy to function. Energy can be supplied either directly to the ion pump, primary active transport, or indirectly by coupling pump-action to an ionic gradient that is actively maintained, secondary active transport. Active transport is encountered commonly in gut mucosa, the liver, renal tubules and the blood–brain barrier.

Na^+/K^+ ATPase is an example of primary active transport – the energy in the high-energy phosphate bond is lost as the molecule is hydrolysed, with concurrent ion transport against the respective concentration gradients. It is an example of an antiport, as sodium moves in one direction and potassium in the opposite direction. The Na^+/amino acid symport (substances moved in the same direction) in the mucosal cells of the small bowel or on the luminal side of the proximal renal tubule is an example of secondary active transport. Here, amino acids will only cross the mucosal cell membrane when Na^+ is bound to the carrier protein and moves down its concentration gradient (which is generated using Na^+/K^+ ATPase). So, directly and indirectly, Na^+/K^+ ATPase is central to active transport (Figure 1.4).

Primary active transport proteins include the ABC (ATP-binding cassette) family, which are responsible for transport of essential nutrients into and toxins out of cells. An important protein belonging to this family is the multi-drug resistant protein transporter, also known

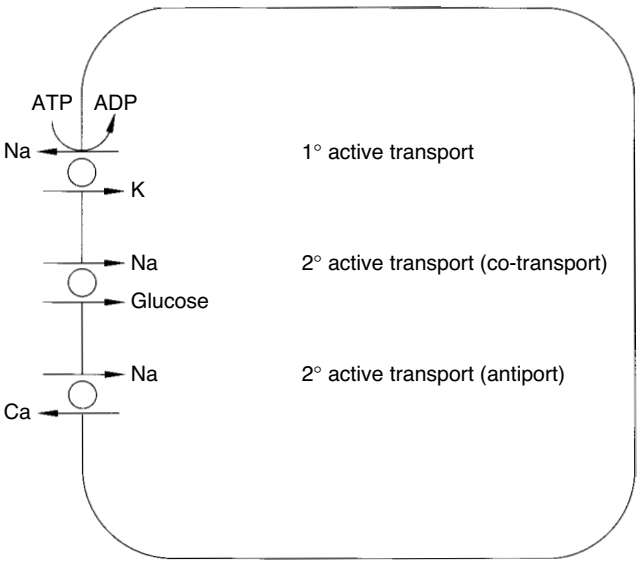

Figure 1.4 Mechanisms of active transport across the cell membrane.

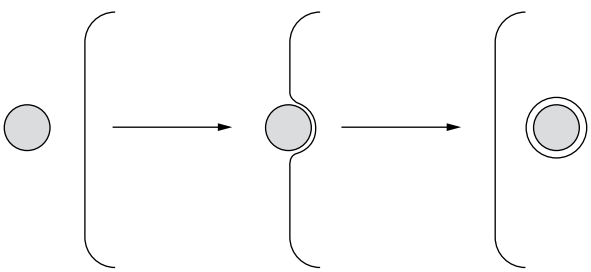

Figure 1.5 Pinocytosis.

as p-glycoprotein (PGP), which is found in gut mucosa and the blood–brain barrier. Many cytotoxic, antimicrobial and other drugs are substrates for PGP and are unable to penetrate the blood–brain barrier.

The anticoagulant dabigatran is a substrate for PGP and co-administration of PGP inhibitors, such as amiodarone and verapamil, will increase dabigatran bioavailability and therefore the risk of adverse haemorrhagic complications. PGP inducers, such as rifampicin, will reduce dabigatran bioavailability and lead to inadequate anticoagulation.

Inhibitors and inducers of PGP are commonly also inhibitors and inducers of CYP3A4 and will interact strongly with drugs that are substrates for both PGP and CYP3A4.

Pinocytosis

Pinocytosis is the process by which an area of the cell membrane invaginates around the (usually large) target molecule and moves it into the cell. The molecule may then be released into the cell or may remain in the vacuole so created, until the reverse process occurs on the opposite side of the cell.

The process is usually used for molecules that are too large to traverse the membrane easily via another mechanism (Figure 1.5).

Factors Influencing the Rate of Diffusion

Molecular Size

The rate of passive diffusion is inversely proportional to the square root of molecular size (Graham's law). In general, small molecules will diffuse much more readily than large ones. The molecular weights of anaesthetic agents are relatively small and anaesthetic agents diffuse rapidly through lipid membranes to exert their effects.

Concentration Gradient

Fick's law states that the rate of transfer across a membrane is proportional to the concentration gradient across the membrane. Thus increasing the plasma concentration of the unbound fraction of the drug will increase its rate of transfer across the membrane and will accelerate the onset of its pharmacological effect. This is the basis of Bowman's principle, applied to the onset of action of non-depolarising muscle relaxants. The less potent the drug, the more required to exert an effect – but this increases the concentration gradient between plasma and active site, so the onset of action is faster.

Ionisation

The lipophilic nature of the cell membrane only permits the passage of the uncharged fraction of any drug. The degree to which a drug is ionised in a solution depends on the molecular structure of the drug and the pH of the solution in which it is dissolved and is given by the Henderson–Hasselbalch equation.

The pK_a is the pH at which 50% of the drug molecules are ionised – thus the concentrations of ionised and unionised portions are equal. The value for pK_a depends on the molecular structure of the drug and is independent of whether it is acidic or basic.

The Henderson–Hasselbalch equation is most simply expressed as:

$$pH = pK_a + \log\left\{\frac{[\text{proton acceptor}]}{[\text{proton donor}]}\right\}.$$

Hence, for an acid (XH), the relationship between the ionised and unionised forms is given by:

$$\text{pH} = \text{pK}_a + \log\left\{\frac{[\text{X}^-]}{[\text{XH}]}\right\},$$

with X^- being the ionised form of an acid.

For a base (X), the corresponding form of the equation is:

$$\text{pH} = \text{pK}_a + \log\left\{\frac{[\text{X}]}{[\text{XH}^+]}\right\},$$

with XH^+ being the ionised form of a base.

Using the terms 'proton donor' and 'proton acceptor' instead of 'acid' or 'base' in the equation avoids confusion and the degree of ionisation of a molecule may be readily established if its pK_a and the ambient pH are known. At a pH below their pK_a weak acids will be more unionised; at a pH above their pK_a they will be more ionised. The reverse is true

for weak bases, which are more ionised at a pH below their pK_a and more unionised at a pH above their pK_a.

Bupivacaine is a weak base with a tertiary amine group in the piperidine ring. The nitrogen atom of this amine group is a proton acceptor and can become ionised, depending on pH. With a pK_a of 8.1, it is 83% ionised at physiological pH.

Aspirin is an acid with a pK_a of 3.0. It is almost wholly ionised at physiological pH, although in the highly acidic environment of the stomach it is essentially unionised, which therefore increases its rate of absorption. However, because of the limited surface area within the stomach more is absorbed in the small bowel.

Lipid Solubility

The lipid solubility of a drug reflects its ability to pass through the cell membrane; this property is independent of the pK_a of the drug as lipid solubility is quoted for the unionised form only. However, high lipid solubility alone does not necessarily result in a rapid onset of action. Alfentanil is nearly seven times less lipid-soluble than fentanyl, yet it has a more rapid onset of action. This is a result of several factors. First, alfentanil is less potent and has a smaller distribution volume and therefore initially a greater concentration gradient exists between effect site and plasma. Second, both fentanyl and alfentanil are weak bases and alfentanil has a lower pK_a than fentanyl (alfentanil = 6.5; fentanyl = 8.4), so that at physiological pH a much greater fraction of alfentanil is unionised and available to cross membranes.

Lipid solubility affects the rate of absorption from the site of administration. Fentanyl is suitable for transdermal application as its high lipid solubility results in effective transfer across the skin. Intrathecal diamorphine readily dissolves into, and fixes to, the local lipid tissues, whereas the less lipid-soluble morphine remains in the cerebrospinal fluid longer, and is therefore liable to spread cranially, with an increased risk of respiratory depression.

Protein Binding

Only the unbound fraction of any drug in plasma is free to cross the cell membrane; drugs vary greatly in the degree of plasma protein binding. In practice, the extent of this binding is of importance only if the drug is highly protein-bound (more than 90%). In these cases, small changes in the bound fraction produce large changes in the amount of unbound drug. In general, this increases the rate at which the drug is metabolised, so a new equilibrium is re-established with little change in free drug concentration. For a very small number of highly protein-bound drugs where metabolic pathways are close to saturation (such as phenytoin) this cannot happen and plasma concentration of the unbound drug will increase and possibly reach toxic levels.

Both albumin and globulins bind drugs; each has many binding sites, the number and characteristics of which are determined by the pH of plasma. In general, albumin binds neutral or acidic drugs (e.g. barbiturates), and globulins (in particular, α_1 acid glycoprotein) bind basic drugs (e.g. morphine).

Albumin has two important binding sites: the warfarin and diazepam sites. Binding is usually readily reversible, and competition for binding at any one site between different drugs can alter the active unbound fraction of each. Binding is also possible at other sites on the molecule, which may cause a conformational change and indirectly influence binding at the diazepam and warfarin sites.

Although α_1 acid glycoprotein binds basic drugs, other globulins are important in binding individual ions and molecules, particularly the metals. Thus, iron is bound to β_1 globulin and copper to α_2 globulin.

Protein binding is altered in a range of pathological conditions. Inflammation changes the relative proportions of the different proteins and albumin concentration falls in any acute infective or inflammatory process. This effect is independent of any reduction in synthetic capacity resulting from liver impairment and is not due to protein loss. In conditions of severe hypoalbuminaemia (e.g. in end-stage liver cirrhosis or burns), the proportion of unbound drug increases markedly such that the same dose will have a greatly exaggerated pharmacological effect. The magnitude of these effects may be hard to estimate and drug dose should be titrated against clinical effect.