

SECTION I Basic principles

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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 specialized 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 specialized 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 (BBB), 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 specialized 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 unionized or ionized form, depending on the pH. The unionized 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.

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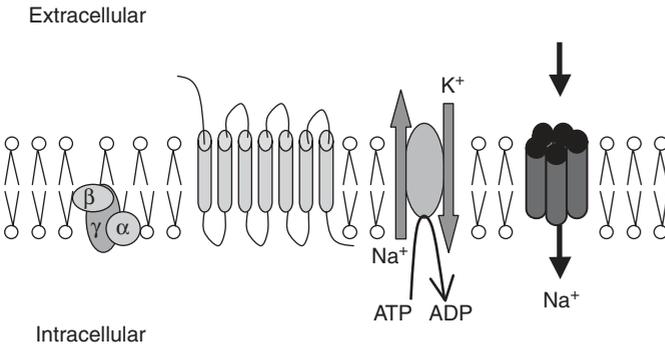


Figure 1.1 Representation of the cell membrane structure. The integral proteins embedded in this phospholipid bilayer are G-protein, G-protein-coupled receptors, transport proteins and ligand-gated ion channels. Additionally, enzymes or voltage-gated ion channels may also be present.

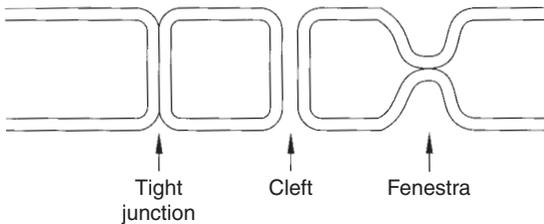


Figure 1.2 Modifications of the general cell membrane structure.

In addition, there are specialized **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 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, depolarization 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 depolarization.

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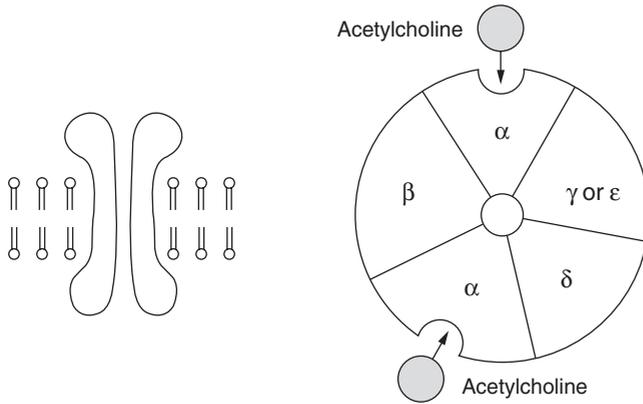


Figure 1.3 The acetylcholine (ACh) receptor has five subunits and spans the cell membrane. ACh binds to the α subunits, causing a conformational change and allowing the passage of small cations through its central ion channel. The ϵ subunit replaces the fetal-type γ subunit after birth once the neuromuscular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

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-depolarizing 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 diffusion alone. An example of this process is the absorption of glucose, a very 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

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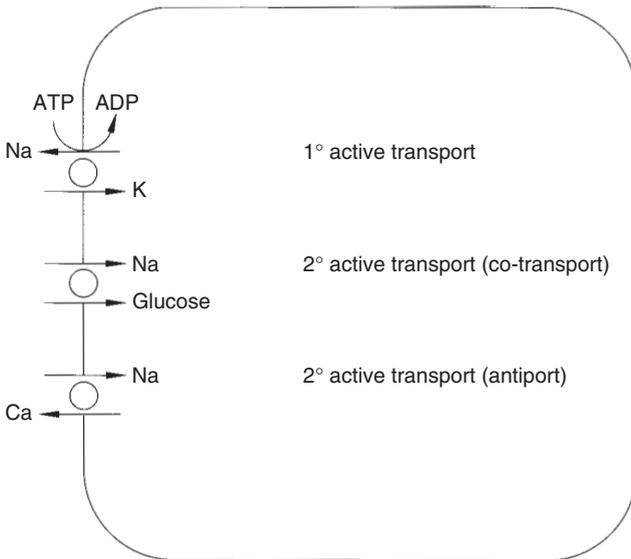


Figure 1.4 Mechanisms of active transport across the cell membrane.

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 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.

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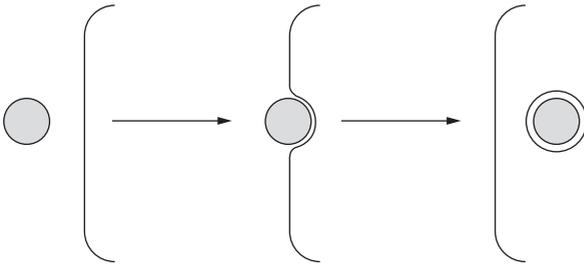


Figure 1.5 Pinocytosis.

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 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-depolarizing 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.

Ionization

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 ionized in a solution depends on the

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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 ionized – thus the concentrations of ionized and unionized 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:

$$pH = pK_a + \log \frac{\text{proton acceptor}}{\text{proton donor}} .$$

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

$$pH = pK_a + \log \frac{X^-}{XH} ,$$

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

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

$$pH = pK_a + \log \frac{X}{XH} ,$$

with XH^+ being the ionized 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 ionization 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 unionized; at a pH above their pK_a they will be more ionized. The reverse is true for weak bases, which are more ionized at a pH below their pK_a and more unionized 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 ionized, depending on pH. With a pK_a of 8.1, it is 83% ionized at physiological pH.

Aspirin is an acid with a pK_a of 3.0. It is almost wholly ionized at physiological pH, although in the highly acidic environment of the stomach it is essentially unionized, 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

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unionized 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 unionized 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 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 drug is metabolized, 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 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

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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.

2

Absorption, distribution, metabolism and excretion

Absorption

Drugs may be given by a variety of routes; the route chosen depends on the desired site of action and the type of drug preparations available. Routes used commonly by the anaesthetist include inhalation, intravenous, oral, intramuscular, rectal, epidural and intrathecal. Other routes, such as transdermal, subcutaneous and sublingual, also can be used. The rate and extent of absorption after a particular route of administration depends on both drug and patient factors.

Not all drugs need to reach the systemic circulation to exert their effects, for example, oral vancomycin used to treat pseudomembranous colitis; antacids also act locally in the stomach. In such cases, systemic absorption may result in unwanted side effects.

Intravenous administration provides a direct, and therefore more reliable, route of systemic drug delivery. No absorption is required, so plasma levels are independent of such factors as gastrointestinal (GI) absorption and adequate skin or muscle perfusion. However, there are disadvantages in using this route. Pharmacological preparations for intravenous therapy are generally more expensive than the corresponding oral medications, and the initially high plasma level achieved with some drugs may cause undesirable side effects. In addition, if central venous access is used, this carries its own risks. Nevertheless, most drugs used in intensive care are given by intravenous infusion this way.

Oral

After oral administration, absorption must take place through the gut mucosa. For drugs without specific transport mechanisms, only unionized drugs pass readily through the lipid membranes of the gut. Because the pH of the GI tract varies along its length, the physicochemical properties of the drug will determine from which part of the GI tract the drug is absorbed.

Acidic drugs (e.g. aspirin) are unionized in the highly acidic medium of the stomach and therefore are absorbed more rapidly than basic drugs. Although weak bases (e.g. propranolol) are ionized in the stomach, they are relatively unionized in the duodenum, so are absorbed from this site. The salts of permanently charged drugs (e.g. vecuronium, glycopyrrolate) remain ionized at all times and are therefore not absorbed from the GI tract.

In practice, even acidic drugs are predominantly absorbed from the small bowel, as the surface area for absorption is so much greater due to the presence of mucosal villi.

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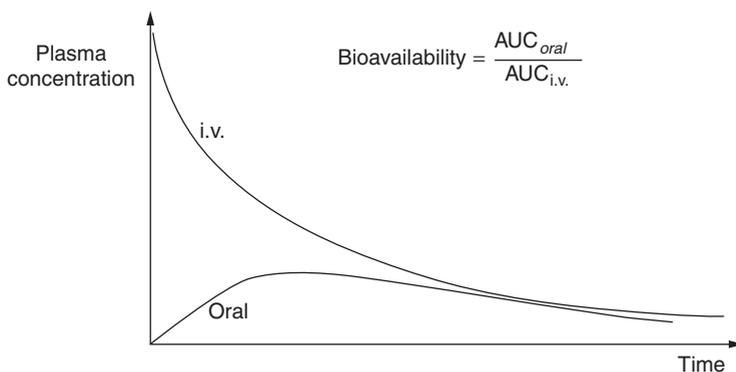


Figure 2.1 Bioavailability may be estimated by comparing the areas under the curves.

However, acidic drugs, such as aspirin, have some advantages over basic drugs in that absorption is initially rapid, giving a shorter time of onset from ingestion, and will continue even in the presence of GI tract stasis.

Bioavailability

Bioavailability is generally defined as the fraction of a drug dose reaching the systemic circulation, compared with the same dose given intravenously (i.v.). In general, the oral route has the lowest bioavailability of any route of administration. Bioavailability can be found from the ratio of the areas under the concentration–time curves for an identical bolus dose given both orally and intravenously (Figure 2.1).

Factors influencing bioavailability

- *Pharmaceutical preparation* – the way in which a drug is formulated affects its rate of absorption. If a drug is presented with a small particle size or as a liquid, dispersion is rapid. If the particle size is large, or binding agents prevent drug dissolution in the stomach (e.g. enteric-coated preparations), absorption may be delayed.
- *Physicochemical interactions* – other drugs or food may interact and inactivate or bind the drug in question (e.g. the absorption of tetracyclines is reduced by the concurrent administration of Ca^{2+} such as in milk).
- *Patient factors* – various patient factors affect absorption of a drug. The presence of congenital or acquired malabsorption syndromes, such as coeliac disease or tropical sprue, will affect absorption, and gastric stasis, whether as a result of trauma or drugs, slows the transit time through the gut.
- *Pharmacokinetic interactions and first-pass metabolism* – drugs absorbed from the gut (with the exception of the buccal and rectal mucosa) pass via the portal vein to the liver where they may be subject to first-pass metabolism. Metabolism at either the gut wall (e.g. glyceryl trinitrate (GTN)) or liver will reduce the amount reaching the circulation.