1.1 Principles of clinical pharmacology

Pharmacology is defined as the study of the effects of drugs on the function of a living organism. It is an integrative discipline that tackles drug/compound behaviours in varied physiological systems and links these to cellular and molecular mechanisms of action.

As a scientific endeavour, pharmacology evolved from the early identification of therapeutic properties of natural compounds, with herbal medicines and relatively complex pharmacopoeias widely used in early cultures. Despite this, lack of understanding of the physiological, pathological, and chemical processes governing the human body prevented the early establishment of pharmacology as a scientific discipline. Since then, **pharmacology** has progressed to be considered a fully developed integrative science that employs techniques and theories from various disciplines, such as chemistry, biochemistry, genomics, medicinal chemistry, physiology, and cellular and molecular biology. Collectively, these are applied to study disease causality and the relevant mechanistic action of compounds, to establish new treatments.

In the last 100 years, the importance of clinical pharmacology has increased in line with the scientific and technological advances in biomedical research. Benefits gained from molecular and cellular approaches have enabled a more comprehensive analysis of drugs and their actions in functional context. Now, *clinical pharmacology and therapeutics* encompass the discovery, development, regulation, and application of drugs in a process that integrates scientific research with clinical practice to better treat illness and preserve health.

Part 1: principles of pharmacology

Within this textbook the principles of pharmacology are discussed by therapeutic area so that the reader can link disease pathophysiology, drug mechanism, and modern prescribing behaviours for conditions commonly seen in clinical practice. There are, however, fundamental concepts that are universal in understanding the interaction between drugs and their 'targets', including receptor pharmacology, genomic pharmacology, and pharmacokinetics.

Receptor pharmacology

The pharmacological receptor models preceded by many years the knowledge of the receptor as an entity. It was not until the last 150 years that a series of contributions from many notable biologists and chemists established the principles that founded modern day pharmacology. They produced a significant paradigm shift in therapeutics, where empirical descriptors of the activities observed (heating, cooling, moistening, emetic, etc.) were replaced by the concept of a 'target'. After more than a century, the basic receptor concept is still the foundation of biomedical research and drug discovery.

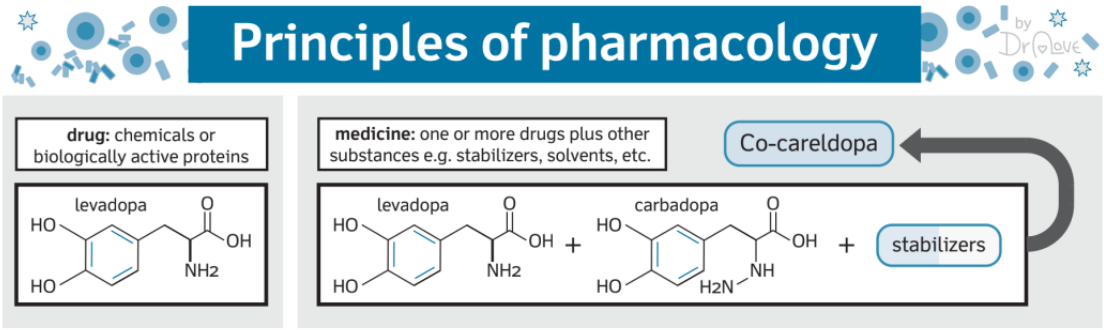

Figure 1.1 Drug or medicine.

Useful Definitions

- *Drug*: a chemical (or biological) substance of recognized structure that produces a biological effect when administered to a living organism. Therapeutic actions are produced through interaction with targets on the cell surface or within the cells, leading to altered cell function, restoring disrupted tissue homeostasis and attenuating disease. The term 'drug' mostly refers to small molecules or biologically active proteins (e.g. monoclonal antibodies) with a known role in therapy or in the context of drug theory. Experimentally used chemicals are more commonly termed compounds, ligands, or new chemical entities (NCE)
- *Medicines*: normally includes one (or more) drugs, plus other substances formulated to improve drug bioavailability and administration (e.g. solvents, preservatives, stabilizing agents, etc.). Substances that are endogenously produced are only considered a medicine when they are exogenously administered, e.g. adrenaline. Medicines used within clinical trials prior to gaining a license are termed investigational medicinal products (IMPs) or investigational new drugs (Figure 1.1).
- *Targets*: defined therapeutic entities producing concentration-dependent effects by interacting with receptive elements in tissues.
- *Receptors*: a recognition site within a cell that is connected to a signal transduction event, and represents the location by which the compound or chemical entities can produce a response in cell physiology.

Lock and key

The ground-breaking concept that all therapeutic agents act by targeting molecular entities comes from the work of Ehrlich and Langley in the early twentieth century, developing the 'lock and key' hypothesis for drug action. In this model, a drug/compound acts as a *ligand* (L) and interacts with a *receptive molecule* (R; drug target) in a reversible manner, forming a receptor–ligand complex (R/L), with a functional consequence that allows the modulation of cell function to preserve/restore tissue homeostasis (Figure 1.2).

Occupancy theory model

The improved understanding of receptor function, promoted the development of the occupancy theory model (Figure 1.2), in which a ligand acts as an *agonist* when it induces a tissue response that is a function of the number of receptors occupied. This concept assumed that the formation of an R/L complex was reversible, and that all receptors were functionally equivalent and had the ability to bind the ligand independently.

In this model, an *antagonist* is a ligand that can occupy the receptor/drug target site, but block the functional response of an agonist. When the functional effect of an antagonist could be overcome by increasing the concentration of the agonist, we define this as a *competitive antagonist*. The blockade of receptor function can also occur via *non-competitive antagonists*, which interact with the receptor at *allosteric sites* other than the site in the receptor that is recognized by the agonist (termed *orthosteric site*).

Intrinsic activity

To incorporate the concept that some agonists are not capable of producing a maximal response even at supramaximal concentrations, the occupancy theory incorporated the notion of *intrinsic activity* (IA) for a ligand. In this, a *full agonist* would have an IA value of 1.0, with zero given to an antagonist.

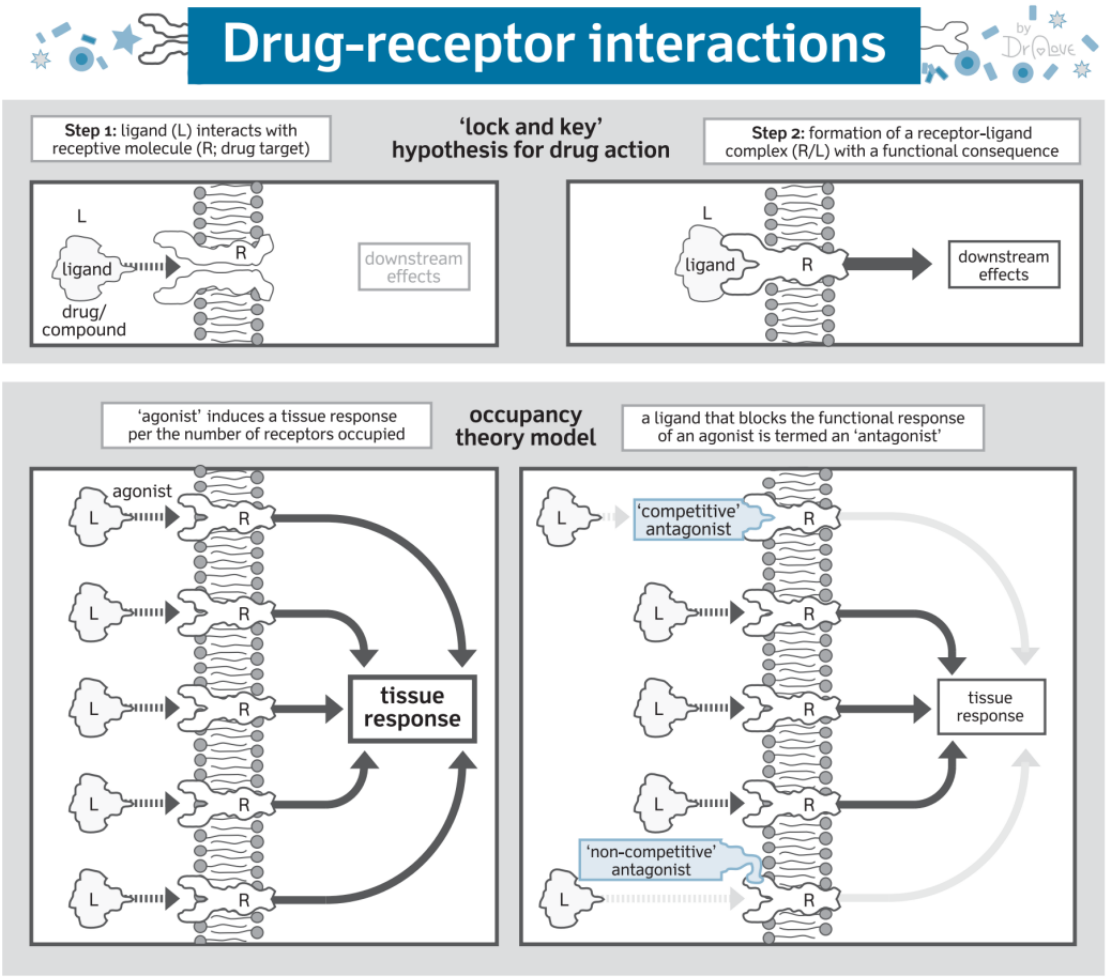

Figure 1.2 Drug–receptor interactions

When compounds that bind to the receptor are only capable of generating a fraction of the response observed with a full agonist, they are termed *partial agonists*, which by definition are also *partial antagonists* (since their receptor occupancy prevents a full agonist response). In the case of agonists that cause a response larger than the assumed 'gold standard' full agonist, these are referred to as *super agonists*.

Affinity

As part of this receptor–ligand theory a binding reaction occurs where free drug [D] and free receptor [R] interact to form a drug–receptor interaction [DR] in a reversible way that is dependent on concentration (Law of Mass Action). The ratio of the rate of the forward reaction and backward reaction define an affinity binding constant, the reciprocal of which is the equilibrium dissociation constant (K_A), which is expressed in mol/L, and is the concentration required to occupy 50% of the binding sites when equilibrium is achieved. When a drug has a low K_A it will have high affinity for its receptor.

$$D + R \leftrightharpoons DR$$

$$K_A = k-1/k+1$$

Useful Definitions

When there is more than one binding site on a receptor or enzyme the Hill coefficient is commonly used to estimate the number of ligand molecules required to bind to a receptor in order to produce a functional outcome. It was empirically applied by A. V. Hill in 1910 to describe oxygen binding to haemoglobin, and then widely used to analyse the binding equilibria in ligand–receptor interactions. In addition to the number of ligand molecules required for a functional outcome, the Hill equation provides a measure of the affinity of the ligand for the receptor. The equation can only accurately estimate the number of binding sites when positive cooperativity exists between the binding of the first and subsequent ligand molecules, i.e. binding for the first site has much lower affinity than subsequent ones. It is thus described as an 'interaction coefficient' that reflects cooperativity, instead of an estimate of binding site numbers. In the case of haemoglobin the Hill coefficient of oxygen binding is 2.3–3.0 demonstrating positive cooperativity. Low affinity for oxygen initially, but with ligand binding the affinity for oxygen increase, hence, a sigmoidal dissociation curve.

Efficacy

The concept of efficacy was initially introduced as a further refinement of the partial agonist model, where a maximal agonist response is observed with only a minor proportion of the total number of receptors occupied. In practice, efficacy has been used to define the magnitude of a response in relation to other compounds, such as agonists, partial agonists or super agonists.

Half maximal effective concentration (EC_{50}) vs effective dose (ED_{50})

In numerical terms, the effect of a drug in a given system can be expressed by the EC_{50}, which is used to describe the concentration at which 50% of maximal agonist effect is observed (Figure 1.3). The action of the ligand may be stimulatory or inhibitory, and generate a dose–response curve that is normally non-linear, but after plotting on a log scale can be transformed into sigmoidal. EC_{50} is generally used for receptor binding assays or cellular/tissue culture biological assays. It should not be confused with ED_{50}, the 'effective dose in 50% of the population'. The ED_{50} is useful to describe observations in experimental models, where the amount of drug at the receptors cannot be predicted (e.g. in vivo or in vitro perfusion studies).

The term IC_{50} (inhibitory concentration) has also been developed to describe the concentration at which a 50% reduction in agonist effect (inhibitory) is observed, after addition of an antagonist. It is often used in radio-ligand binding assays and denotes the functional strength of an antagonist, rather than true affinity.

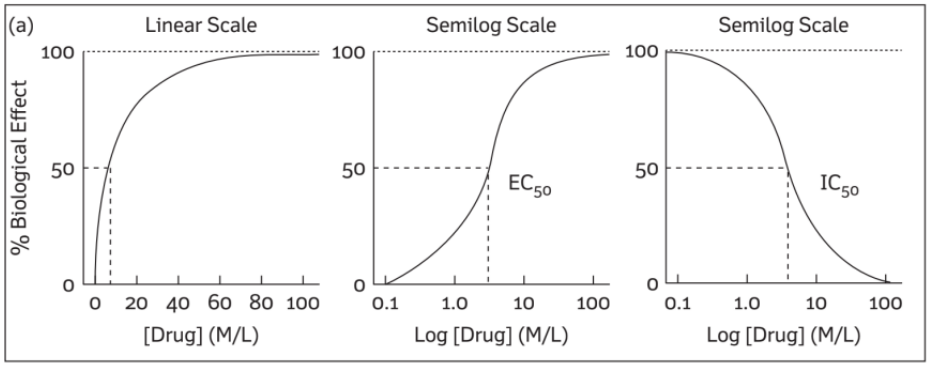

Figure 1.3 Dose–response curve. (a) The dose–response curve plotted on a linear scale (left panel); it is hyperbolic and is described by a Langmuir binding isotherm model (i.e. high concentrations lead to maximum drug effect). Right panel shows drug concentration expressed in logarithmic scale, which converts the plotted dose response from hyperbolic to sigmoid. In this way, the area between 25–75% of the maximum response will be linear and thus better for interpreting drug actions, e.g. EC_{50} and IC_{50}. (b) The inhibition constant (K_i) is an indication of how potent an inhibitor is. While the IC_{50} is more reflective of the functional strength of the inhibitor, the K_i is reflective of the binding affinity. In the equation depicted, [A] is fixed concentration of agonist, EC_{50} represents concentration of agonist resulting in half maximal activation of receptor. The IC_{50} can vary depending on experiment type and condition. K_i is an absolute value and is helpful in determining the likelihood that a particular drug is going to inhibit a particular target.

Therapeutic index

Beyond the therapeutic effect stated by the ED_{50} of a given drug, most drugs have toxic or unwanted side-effects that are dose-related (Type A adverse reactions). These properties can lead to a reluctance to develop and market the product, or non-compliance in the patient population. The therapeutic versus toxic effect can be defined as the therapeutic index (TI) and is indicative of a safety margin. In reality, TI is rarely quoted as a number, being clinically irrelevant because the ED_{50} is highly variable depending on what measures of effectiveness are used, while the LD_{50} (individual/lethal dose required to kill 50% of a population of test animals) does not reflect individual therapeutic toxicity.

$$TI = LD_{50} / ED_{50}$$

Despite this, such safety and efficacy considerations are paramount in the process of developing a drug, and the term 'narrow therapeutic index' is used generically to describe a drug with a narrow margin between effective and toxic levels.

Receptor activation

Two-state model

The concept that receptor activation by an agonist involved a conformational change in the receptor, was implicit in early pharmacological thinking. As such, the simplest theory to account for receptor activation assumes that a receptor exists in one of two conformational states, i.e. a *resting* (or closed) and an *active* (or open) state, with an equilibrium constant that determines the distribution of receptors between the two states. In the case of most receptors, this intrinsic equilibrium favours the inactive conformation and, therefore, receptors tend to be inactive in the absence of ligands.

In this model (Figure 1.4), an *agonist* is a ligand that preferentially binds to the active receptor conformation, using the free energy released to trap a fraction of the receptor molecules in the active state. When the drug has zero affinity for the inactive conformation, the active fraction will eventually exceed the ligand-bound fraction and this ligand is therefore a *full agonist* (e.g. the opioid **methadone**).

This model assumes that *antagonists* (e.g. α- and β-adrenergic blocking agents) bind selectively to the *resting* receptor conformation, shifting receptor equilibrium and thereby reducing receptor activity. This type of drug is also referred to as an *inverse agonist*. In the case of *partial agonists* (e.g. **buprenorphine** on opioid receptors), they should be able to bind to both the active and resting

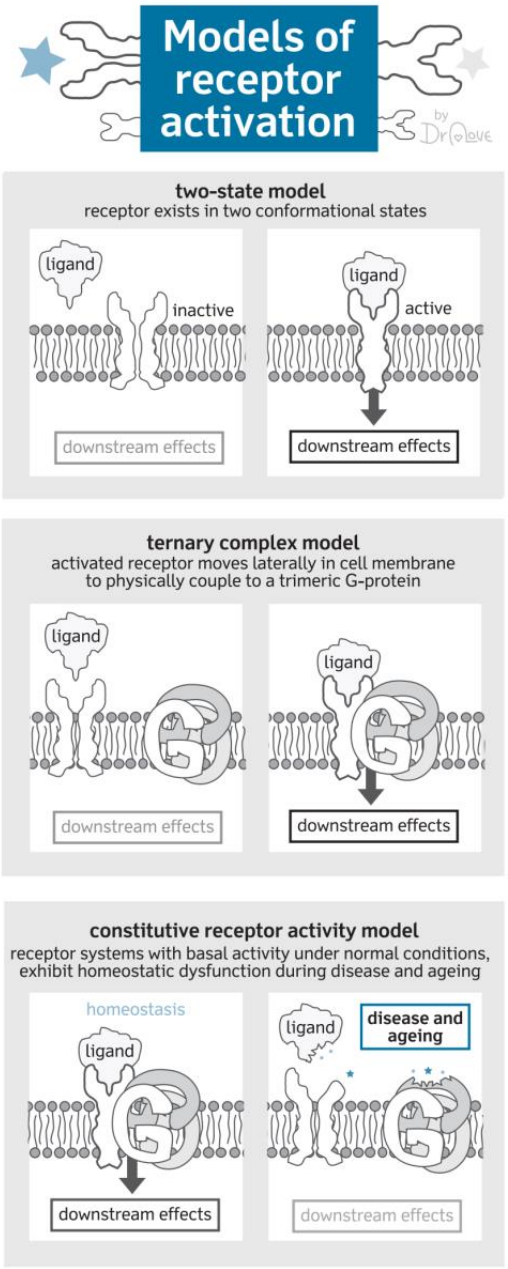

Figure 1.4 Models of receptor activation.

states, but achieve partial activation by having greater affinity for the active conformation. The mostly theoretical assumption of a ligand with perfectly equal affinities for active and resting states, would not change conformational equilibrium and receptor activity. These drugs are

defined as *neutral antagonists*, since they could result in an antagonistic effect in the presence of an agonist.

The use of this type of model of receptor activation is central to the study of ligand-gated ion channels, and although this was not always the case for G protein–coupled receptors, it is now acknowledged that both these major families of receptors can observe the same, two-state mechanism of receptor activation.

Ternary complex model

Allosterism is a change in enzyme activity and conformation, following the binding of a ligand or protein at a site other than the active binding site. The model of allosterism was first defined for ion channels and enzymes, and was then used for receptors, leading to the model of the ternary complex for G protein–coupled receptors. Here, the activated receptor can move laterally in the cell membrane to physically couple to a trimeric G protein. In this mechanistic process the sensitivity of the system is dependent on the availability of an external component (i.e. a G protein). The three-component ternary complex model (Figure 1.4), establishes an equilibrium between the ligand-bound receptor and free G protein; and also among the receptor, ligand, and G protein complex.

Constitutive receptor activity model

This model was formulated to reflect the fact that receptors can also naturally form active complexes, resulting from interactions with other proteins in the absence of any ligand, an event denominated *constitutive activity*. This can occur with both G protein–coupled receptors and ion channels, and can reflect both the basal activity in a normally inactive system, as well as the homeostatic dysfunction observed in disease and ageing. Constitutive receptor activation is linked to allosteric transition in receptor states, where changes in the conformation of a receptor can happen through random thermal occurrences, leading to spontaneous activation.

It has now been suggested that receptors are generally in a constitutively active state that is regulated by other cellular factors in normal homeostatic conditions. This could explain why most drugs acting via receptors can function as antagonists.

Receptor types and drug targets

The receptor concept in its simplest form, i.e. a molecular entity capable of responding to the interaction with a ligand, has gradually expanded to incorporate different types of drug targets/receptors.

Transmembrane ion channels

These are pore-forming membrane proteins that gate the flow of ions across biological membranes (see Figure 1.5). Since phospholipid bilayers act as electrical insulators, ion channels constitute a high conducting, hydrophilic pathway across a membrane. This ion movement controls membrane potential and shapes many regulatory processes dependent on electrical signals and downstream Ca^{2+}.

Transmembrane ion channels have a prominent role in the function of neurons and synapses, making them common targets for organisms that want to arrest the nervous systems of predators and prey (e.g. **tetrodotoxin**, a potent neurotoxin). Their capacity to regulate ion conductance and membrane potential means they are also key components in various cellular processes, including muscle contraction, T-cell activation, pancreatic β-cell insulin release, and epithelial transport. These are frequent targets in drug development.

Hundreds of different ion channels have been identified, and are classified according to structure, gating mechanism, and ion permeability. The latter refers to the selective permeability to ions that is dependent on size and/or charge, producing a very high rate of transport through the channel. Since ions move down their electrochemical gradient (dependent on ion concentration and membrane potential), there is no required input of metabolic energy (as is the case in adenosine triphosphate (ATP)/active transport mechanisms, see 'Transporters').

Depending on the mechanism that triggers the conformational change required for channel gating (opening/closing), ion channels are classified into two main categories:

- *ligand-gated ion channels*, which open in response to ligand molecules binding to the extracellular domain.
- *voltage-gated ion channels*, which open following a conformational alteration triggered by changes in membrane potential.

In addition, *second messengers* can also activate ion channels from the intracellular side of the membrane, while *mechanosensitive* channels open under the influence of stretch, pressure, shear, or displacement, and *temperature-gated* channels can open in response to hot or cold temperatures.

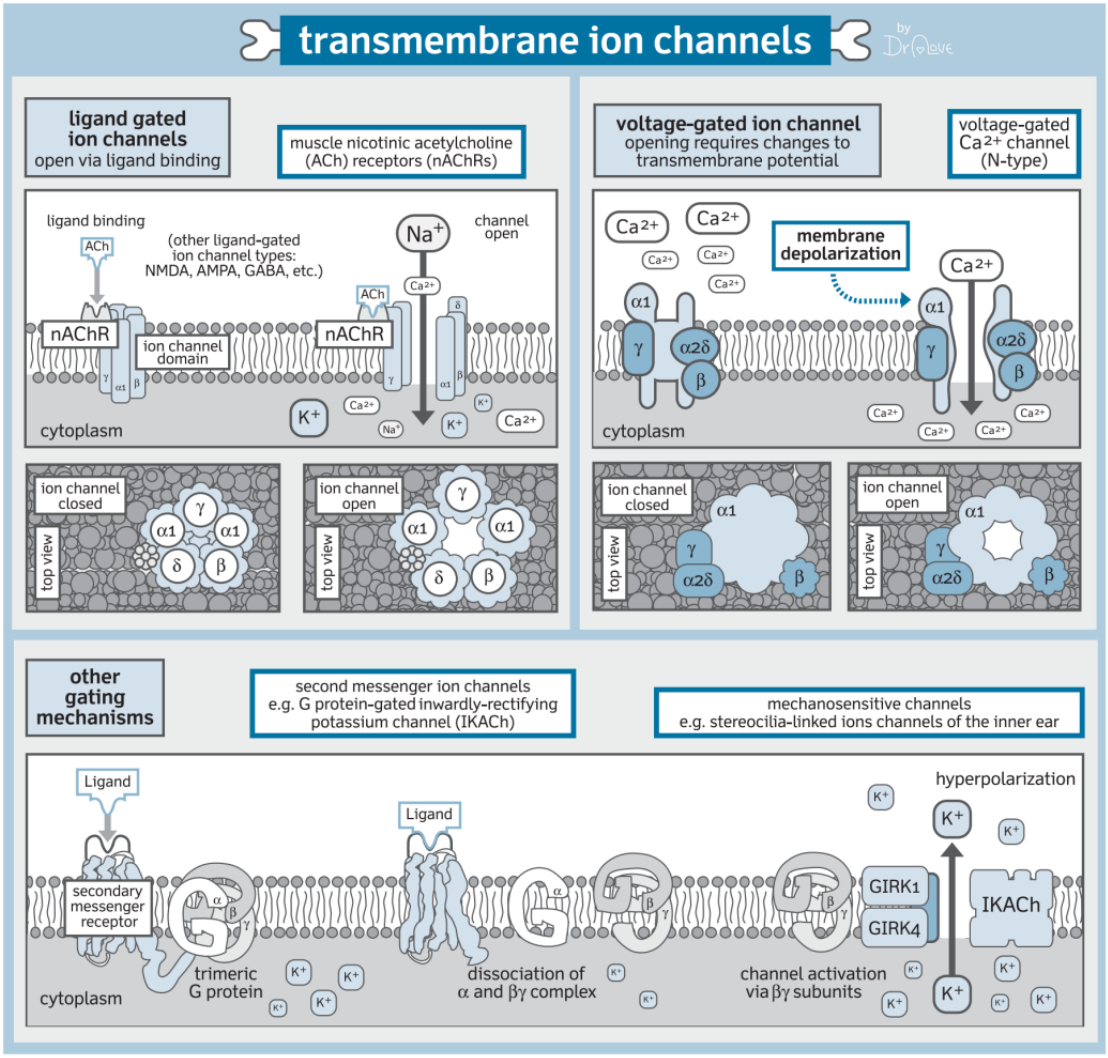

Figure 1.5 Transmembrane ion channels.

Transporters

This group includes membrane-spanning proteins that allow the movement of ions, peptides, small molecules and macromolecules across biological membranes (see Figure 1.6). They are distinguished from channels as the facilitation of movement occurs by physical binding and movement of the substance across the lipid bilayer. The action of transporters can be classified as either:

- *passive*: the transport of a molecule from high to low concentration areas;
- *active*: transport from an area of low to high concentration, which being thermodynamically unfavourable needs to be linked to the energy release of ATP hydrolysis and are thus referred to as ATPases.

Transporters are also classified based on the stoichiometry of the transport process across the membrane. In this way, *uniporters* transport a single molecule at a time, *symporters* transport two different molecules in the same direction simultaneously, and *antiporters* transport two different molecules in opposite directions.

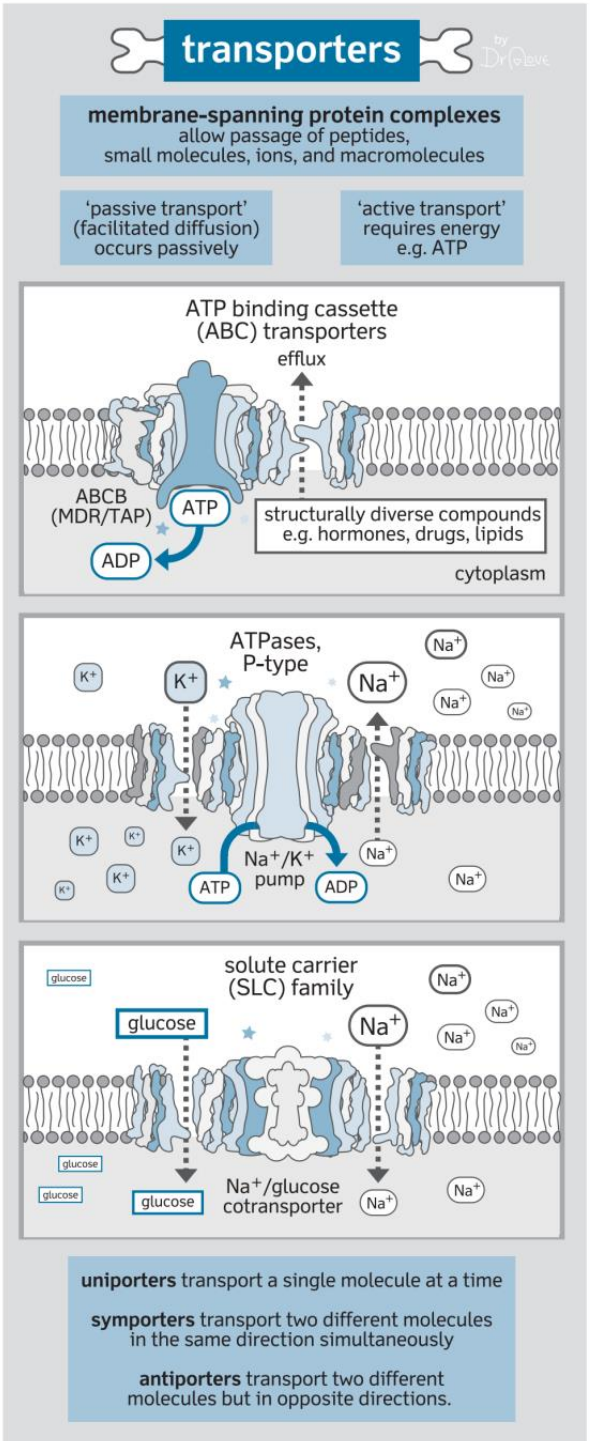

Figure 1.6 Transporters.

7-transmembrane heptahelical G protein–coupled receptors

This constitutes the largest and more diverse group of membrane receptors, with a vast number of functions in the body (see Figure 1.7). It is estimated that between 30 and 50% of drugs available in the market act by targeting this group of receptors. Structurally, they are made by a single polypeptide chain folded in a globular shape with seven segments spanning the entire membrane and intervening loops inside and outside the cell. Their cellular function is accomplished by the capacity to interact with G proteins in the plasma membrane. Binding of the receptor ligand causes a conformational change that triggers the interaction with G proteins.

These specialized heterotrimeric proteins have three different subunits (α, β, and Υ), with two attached to the plasma membrane by lipid anchors. When inactive, a guanosine diphosphate (GDP) attaches to the α subunit and the G protein is bound to the receptor complex. The conformational change provoked by binding of the ligand to the receptor, activates the G protein so that a guanosine triphosphate (GTP) replaces the GDP bound to the α subunit. GTP binding leads to the dissociation of the G protein into the GTP-bound α subunit and the β–Υ dimer, and both remain attached to the membrane. Membrane diffusion allows the active α subunit to interact with other membrane proteins and thus regulate signalling processes inside the cell. The hydrolysis of GTP back to GDP, promotes the reassembly of the heterotrimeric complex, which is then ready to bind an inactive transmembrane receptor complex.

Nuclear receptors

This includes a superfamily of ligand-activated transcription factors that can regulate the expression of specific genes; thus, controlling a variety of cellular processes in

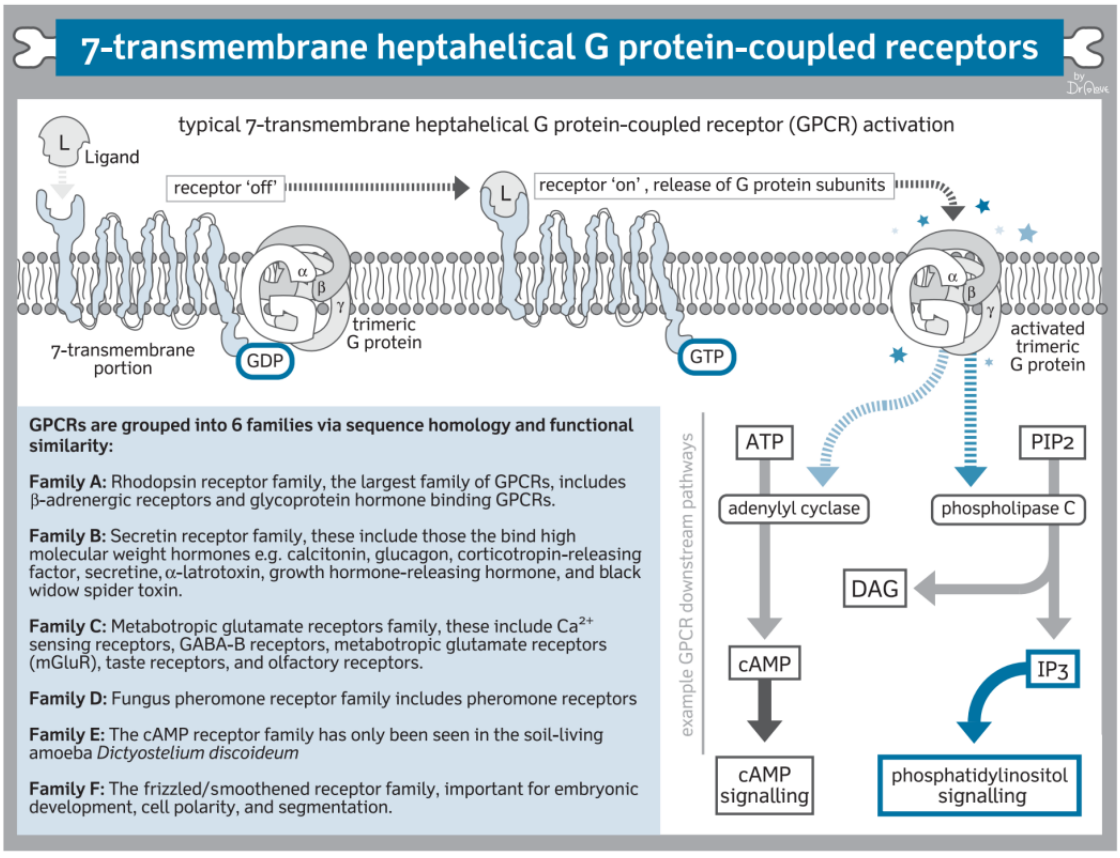

Figure 1.7 7-transmembrane heptahelical G protein-coupled receptors.

differentiation/development, proliferation, metabolism and cell homeostasis (see Figure 1.8). In structural terms, the superfamily is characterized by the presence of an N-terminal transactivation domain, a highly conserved DNA-binding domain, and a C-terminal ligand-binding domain. As well as being activated through ligand-binding, nuclear receptor activity can also be regulated by numerous signalling molecules that result in receptor phosphorylation or other post-translational modifications. These commonly target the transactivation domain in the N-terminus of the protein.

Nuclear receptors are found within the cells; therefore, ligands capable of binding and activating them are lipophilic substances, i.e. steroid hormones. Some of the members of this superfamily have no fully determined ligands, such as metabolic intermediates or xenobiotic endocrine disruptors.

The superfamily can be classified into two broad classes according to their mechanism of action and subcellular distribution when not bound to the ligand

- *Type I*: nuclear receptors bind to their ligands in the cytoplasmic compartment, resulting in dissociation

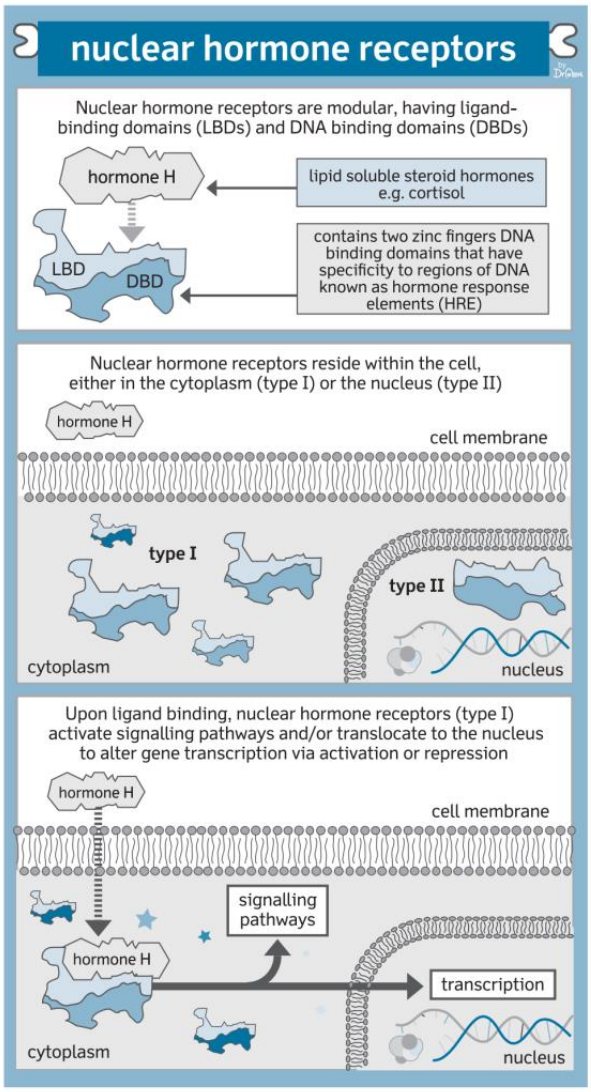

Figure 1.8 Nuclear hormone receptors.

from heat shock proteins, homodimerization, and translocation into the nucleus. This class includes members such as androgen receptors, oestrogen receptors, and glucocorticoid receptors.

- *Type II*: are located in the nucleus, regardless of ligand-binding status, and bind to the DNA as heterodimers. Examples of receptors that belong to this group are the retinoic acid receptor and thyroid hormone receptor.

Catalytic receptors

These are membrane proteins, usually dimeric, composed of binding and functional domains in one polypeptide chain and includes the cytokine, receptor tyrosine kinase and phosphatase, receptor serine/threonine kinase, and tumour necrosis factor families. The *ligand-binding domain* is located on the extracellular side of the plasma membrane, with a transmembrane-spanning domain comprised of 20–25 amino acids that separates the *functional domain* on the intracellular side. This functional domain has catalytic activity or is capable of interacting with specific enzymes leading to the activation of signalling pathways.

The endogenous agonists are normally peptides or proteins that induce dimerization, which is the functional conformation of the receptor. Classification is based on the particular function of the enzymatic portion of the receptor, and this is depicted in Figure 1.9.

Enzymes

This group, which includes serine/threonine kinase super families, act as efficient catalysts of biochemical reactions by providing an alternative reaction pathway with lower energy of activation. Although they take part in the reaction, enzymes remain unchanged, only altering the rate of the reaction, not the direction of the equilibrium.

The molecular structure of enzymes makes them very specific catalysts for a wide range of reactions. In most cases, enzymes are associated with a non-protein part called a *co-factor*, which includes permanently bound organic groups (prosthetic groups), temporarily bound cations that provide positive charges to the active site and organic molecules. The latter are not permanently bound to the enzyme, but can facilitate its function by associating temporarily with the enzyme-substrate complex.

Enzymes have an *active site*, which has the shape and chemical groups to bind to the reacting molecule, or *substrate*. The catalytic activity of enzymes can be severely affected by changes in temperature and pH. A rise in the former, can increase kinetic energy and promote the chances of successful collision of enzyme and substrate, thus increasing the reaction rate. Beyond this optimal temperature, intra- and intermolecular bonds can be broken and enzymes begin to denature. Changes in pH can also affect the molecular structure of the protein and thus each enzyme has a narrow range for optimal activity (optimal pH).

The block or distortions of the active site by substances can reduce/stop the catalytic activity of enzymes. These are called enzyme inhibitors and can be divided as *competitive* (active site–directed because they bind to the active site) or *non-competitive* (non-active site–directed because they interact with other parts of the enzyme molecule). Statins are a current example of competitive inhibitors that target HMG-CoA reductase controlling cholesterol biosynthesis. **Nifedipine**, on the contrary, acts as a non-competitive inhibitor of the CYP2C9 enzyme.

The type of binding also divides enzyme inhibitors into reversible or irreversible types. The latter usually changes the enzyme chemically (e.g. covalent bonds), modifying amino acids required for the enzymatic reaction. Reversible inhibitors bind non-covalently, whether to the enzyme, the enzyme-substrate complex or both.

Emerging drug targets

Although not technically grouped as a receptor type, biological research has started to incorporate a variety of new targets as potential options for therapy. These include messenger RNAs, micro- and short-interfering RNAs, non-coding functional elements in the DNA and antibodies. For example, there are many drug-discovery programmes that focus on miRNA-based therapeutics. This is based on numerous studies that show dramatic changes in miRNA populations in many pathological processes. As such, targeting specific miRNAs with modified oligonucleotides [i.e. locked nucleic acid (LNA)] could provide a viable option for therapy. In addition, monoclonal antibody therapy constitutes another emerging field in clinical research, used to specifically target proteins, either to directly block an abnormal function or to stimulate the patient's immune system. In recent years, the use of monoclonal antibodies for cancer therapy has evolved as a powerful new treatment option, with immunomodulatory antibodies also achieving remarkable clinical success. In particular, the development of molecular techniques that can alter antibody pharmacokinetics, effector function, size, and immunogenicity have emerged as crucial factors in the establishment of antibody-based therapies.

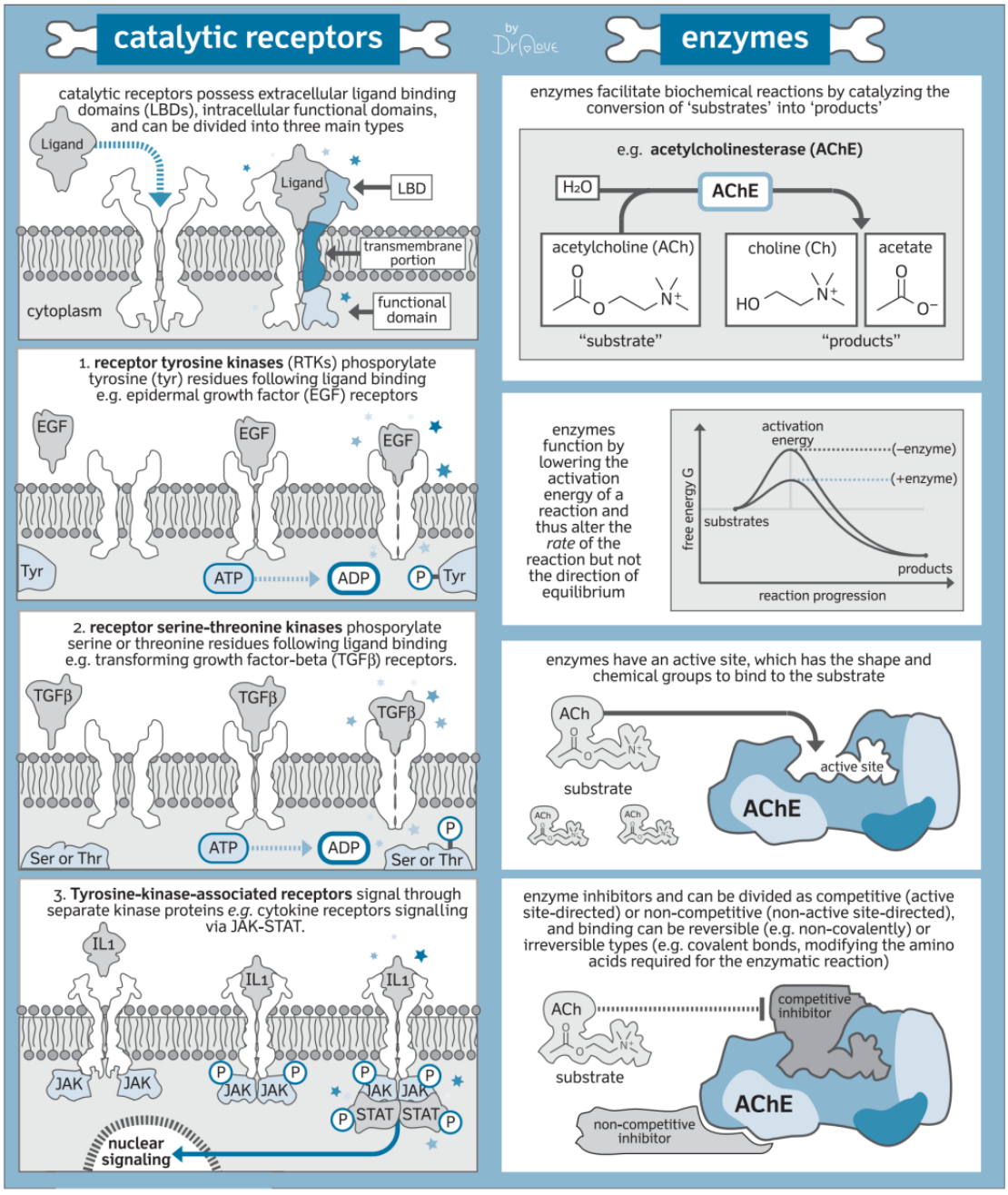

Figure 1.9 Catalytic receptors enzymes.