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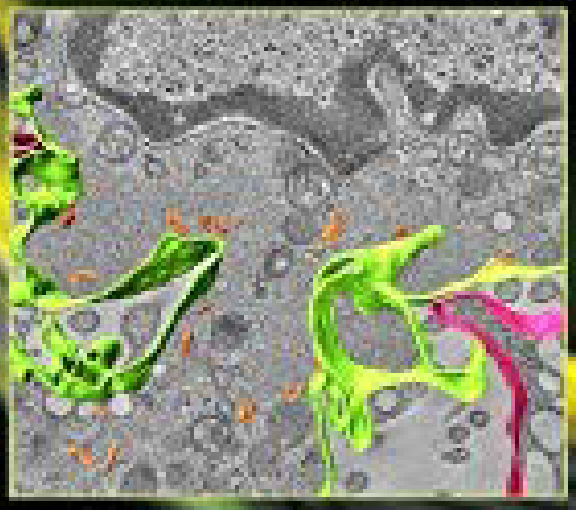

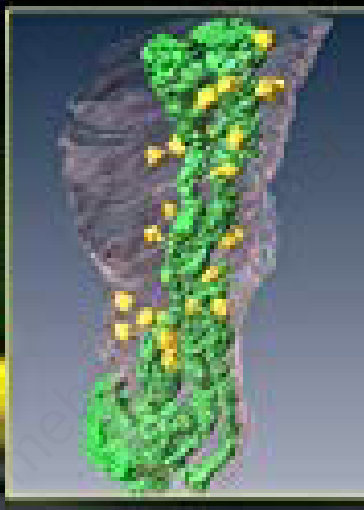

RANG & DALE'S Pharmacology

NINTH EDITION

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Pharmacology

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Rang and Dale's Pharmacology Ninth Edition Preface

In this edition, as in its predecessors, we set out to explain what drugs do in terms of the mechanisms by which they act. This entails analysis not only at the cellular and molecular level, where knowledge and techniques are advancing rapidly, but also at the level of physiological mechanisms and pathological disturbances. Pharmacology has its roots in therapeutics, where the aim is to ameliorate the effects of disease, so we have attempted to make the link between effects at the molecular and cellular level and the range of beneficial and adverse effects that humans experience when drugs are used for therapeutic or other reasons. Therapeutic agents have a high rate of obsolescence. In the decade 2008 to 2017, 301 new drugs gained regulatory approval for use as therapeutic agents. The majority exploit the same molecular targets as drugs already in use. Knowledge of the mechanisms of action of the class of drugs to which a new agent belongs provides a good starting point for understanding and using a new compound intelligently.

Significantly, however, one-third of these new arrivals are 'first-in-class' drugs. That is, they act on novel molecular targets not previously exploited for therapeutic purposes, and are therefore likely to produce effects not previously described. Not all will succeed clinically, but some will stimulate the development of improved follow-up compounds of the same type. Furthermore, about a quarter of the new compounds are 'biopharmaceuticals' – mainly proteins produced by bioengineering rather than synthetic chemistry. These are growing in importance as therapeutic agents, and generally have characteristics somewhat different from conventional drugs and are covered in a revised chapter. The very high rate of innovation in drug discovery is a recent – and very welcome – change, due in large part to the rapid advances in molecular and cell biology that have stemmed from the sequencing of the human genome in 2003. We have tried to strike a balance between the need to keep up with these modern developments and the danger of information overload. Our emphasis is on explaining the general principles underlying drug action, which apply to old and new alike, and to describe in more detail the actions and mechanisms of familiar, established drugs, while including references that cover modern and future developments.

Pharmacology is a lively scientific discipline in its own right, with an importance beyond that of providing a basis for the use of drugs in therapy, and we aim to provide a good background, not only for future doctors but also for scientists in other disciplines who need to understand how drugs act. We have, therefore, where appropriate, described how drugs are used as probes for elucidating cellular and physiological functions, to improve our understanding of how the human body functions normally and what goes wrong with it in disease, even when the compounds have no clinical use. Besides therapeutic applications, drugs have other impacts on society, which we cover in chapters on psychoactive drugs, drug abuse, and the use of drugs in sport.

Names of drugs and related chemicals are established through usage and sometimes there is more than one name in common use. For prescribing purposes, it is important to use standard names, and we follow, as far as possible, the World Health Organization's list of recommended international non-proprietary names (rINN). Sometimes these conflict with the familiar names of drugs (e.g. the endogenous mediator prostaglandin I_2 – the standard name in the scientific literature – becomes 'epoprostenol' – a name unfamiliar to most scientists – in the rINN list). In these cases, we generally adopt conventional scientific nomenclature. Sometimes English and American usage varies (as with adrenaline/epinephrine and noradrenaline/norepinephrine). Adrenaline and noradrenaline are the official names in EU member states and are used in this book.

Drug action can be understood only in the context of what else is happening in the body. So, at the beginning of most chapters, we briefly discuss the physiological and biochemical processes relevant to the action of the drugs described in that chapter. We have included the chemical structures of drugs only where this information helps in understanding their pharmacological and pharmacokinetic characteristics, since chemical structures are readily available for reference online.

The overall organisation of the book has been retained, with sections covering: (1) the general principles of drug action; (2) the chemical mediators and cellular mechanisms with which drugs interact in producing their therapeutic effects; (3) the action of drugs on specific organ systems; (4) the action of drugs on the nervous system; (5) the action of drugs used to treat infectious diseases and cancer; and (6) a range of special topics such as adverse effects, non-medical uses of drugs, etc. This organisation reflects our belief that drug action needs to be understood, not just as a description of the effects of individual drugs and their uses, but as a chemical intervention that perturbs the network of chemical and cellular signalling that underlies the function of any living organism. In addition to updating each chapter, we have added new material on biopharmaceuticals, and on personalised medicine, topics of particular current interest. Additional current material on cognition-enhancing drugs has been included in Chapter 48.

Despite the fact that pharmacology, like other branches of biomedical science, advances steadily, with the acquisition of new information, the development of new concepts and the introduction of new drugs for clinical use, we have avoided making the ninth edition any longer than its predecessor by cutting out dated and obsolete material, and have made extensive use of small print text to cover more specialised and speculative information that is not essential to understanding the key message, but will, we hope, be helpful to students seeking to go into greater depth. In selecting new material for inclusion, we have taken into account not only new agents but also recent extensions of basic knowledge that presage further drug development. And, where possible, we have given a brief outline of new treatments in the pipeline. Reference lists are largely restricted to guidance on further reading, together with review articles that list key original papers.

Finally, we hope that we have conveyed something of our own enthusiasm for the science and importance of pharmacology in the modern world.

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1 What is pharmacology?

OVERVIEW

In this introductory chapter we explain how pharmacology came into being and evolved as a scientific discipline, and describe the present-day structure of the subject and its links to other biomedical sciences. The structure that has emerged forms the basis of the organisation of the rest of the book. Readers in a hurry to get to the here-and-now of pharmacology can safely skip this chapter.

WHAT IS A DRUG?

For the purposes of this book, a drug can be defined as *a chemical substance of known structure, other than a nutrient or an essential dietary ingredient,*[1] *which, when administered to a living organism, produces a biological effect.*

A few points are worth noting. Drugs may be synthetic chemicals, chemicals obtained from plants or animals, or products of biotechnology (biopharmaceuticals). A *medicine* is a chemical preparation, which usually, but not necessarily, contains one or more drugs, administered with the intention of producing a therapeutic effect. Medicines usually contain other substances (excipients, stabilisers, solvents, etc.) besides the active drug, to make them more convenient to use. To count as a drug, the substance must be administered as such, rather than released by physiological mechanisms. Many substances, such as insulin or thyroxine, are endogenous hormones but are also drugs when they are administered intentionally. Many drugs are not used commonly in medicine but are nevertheless useful research tools. The definition of drug also covers toxins, which again are not usually administered in the clinic but nonetheless are critical pharmacological tools. In everyday parlance, the word *drug* is often associated with psychoactive substances and addiction – unfortunate negative connotations that tend to bias uninformed opinion against any form of chemical therapy. In this book we focus mainly on drugs used for therapeutic purposes but also describe psychoactive drugs and provide important examples of drugs used as experimental tools. Poisons fall strictly within the definition of drugs, and indeed 'all drugs are poisons… it is only the dose which makes a thing poison' (an aphorism credited to Paracelsus, a 16th century Swiss physician); conversely, poisons may be effective therapeutic agents when administered in sub-toxic doses. Botulinum toxin (Ch. 14) provides a striking example: it is the most potent poison known in terms of its lethal dose, but is widely used both medically and cosmetically. General aspects of harmful effects of drugs are considered in Chapter 58. Toxicology is the study of toxic effects of chemical substances (including drugs), and toxicological testing is undertaken on new chemical entities during their development as potential medicinal products (Ch. 60), but the subject is not otherwise covered in this book.

ORIGINS AND ANTECEDENTS

Pharmacology can be defined as the study of the effects of drugs on the function of living systems. As a science, it was born in the mid-19th century, one of a host of new biomedical sciences based on principles of experimentation rather than dogma that came into being in that remarkable period. Long before that – indeed from the dawn of civilisation – herbal remedies were widely used, pharmacopoeias were written, and the apothecaries' trade flourished. However, nothing resembling scientific principles was applied to therapeutics, which was known at that time as *materia medica*.[2] Even Robert Boyle, who laid the scientific foundations of chemistry in the middle of the 17th century, was content, when dealing with therapeutics (*A Collection of Choice Remedies*, 1692), to recommend concoctions of worms, dung, urine and the moss from a dead man's skull. The impetus for pharmacology came from the need to improve the outcome of therapeutic intervention by doctors, who were at that time skilled at clinical observation and diagnosis but broadly ineffectual when it came to treatment.[3] Until the late 19th century, knowledge of the normal and abnormal functioning of the body was too rudimentary to provide even a rough basis for understanding drug effects; at the same time, disease and death were regarded as semi-sacred subjects, appropriately dealt with by authoritarian, rather than scientific, doctrines. Clinical practice often displayed an obedience to authority and ignored what appear to be easily ascertainable facts. For example, cinchona bark was recognised as a specific and effective treatment for malaria, and a sound protocol for its use was laid down by Lind in 1765. In 1804, however, Johnson declared it to be unsafe until the fever had subsided, and he recommended instead the use of large doses of calomel (mercurous chloride) in the early stages – a murderous piece of advice that was slavishly followed for the next 40 years.

[1]Like most definitions, this one has its limits. For example, there are a number of essential dietary constituents, such as iron and various vitamins, that are used as medicines. Furthermore, some biological products (e.g. **epoietin**) show batch-to-batch variation in their chemical constitution that significantly affects their properties. There is also the study of pharmaceutical-grade nutrients or 'nutraceuticals'.

[2]The name persists today in some ancient universities, being attached to chairs of what we would call clinical pharmacology.

[3]Oliver Wendell Holmes, an eminent physician, wrote in 1860: '[I] firmly believe that if the whole materia medica, as now used, could be sunk to the bottom of the sea, it would be all the better for mankind and the worse for the fishes' (see Porter, 1997).

The motivation for understanding what drugs can and cannot do came from clinical practice, but the science could be built only on the basis of secure foundations in physiology, pathology and chemistry. It was not until 1858 that Virchow proposed the cell theory. The first use of a structural formula to describe a chemical compound was in 1868. Bacteria as a cause of disease were discovered by Pasteur in 1878. Previously, pharmacology hardly had the legs to stand on, and we may wonder at the bold vision of Rudolf Buchheim, who created the first pharmacology institute (in his own house) in Estonia in 1847.

In its beginnings, before the advent of synthetic organic chemistry, pharmacology concerned itself exclusively with understanding the effects of natural substances, mainly plant extracts – and a few (mainly toxic) chemicals such as mercury and arsenic. An early development in chemistry was the purification of active compounds from plants. Friedrich Sertürner, a young German apothecary, purified morphine from opium in 1805. Other substances quickly followed, and, even though their structures were unknown, these compounds showed that chemicals, not magic or vital forces, were responsible for the effects that plant extracts produced on living organisms. Early pharmacologists focused most of their attention on such plant-derived drugs as quinine, digitalis, atropine, ephedrine, strychnine and others (many of which are still used today and will have become old friends by the time you have finished reading this book).[4]

PHARMACOLOGY IN THE 20TH AND 21ST CENTURIES

Beginning in the 20th century, the fresh wind of synthetic chemistry began to revolutionise the pharmaceutical industry, and with it the science of pharmacology. New synthetic drugs, such as barbiturates and local anaesthetics, began to appear, and the era of antimicrobial chemotherapy began with the discovery by Paul Ehrlich in 1909 of arsenical compounds for treating syphilis. Around the same time, William Blair-Bell was world renowned for his pioneering work at Liverpool in the treatment of breast cancers with another relatively poisonous agent, lead colloid mixtures. The thinking was that yes, drugs were toxic, but they were slightly more toxic to a microbe or cancer cell. This early chemotherapy has laid the foundations for much of the antimicrobial and anticancer therapies still used today. Further breakthroughs came when the sulfonamides, the first antibacterial drugs, were discovered by Gerhard Domagk in 1935, and with the development of penicillin by Chain and Florey during the Second World War, based on the earlier work of Fleming.

These few well-known examples show how the growth of synthetic chemistry, and the resurgence of natural product chemistry, caused a dramatic revitalisation of therapeutics in the first half of the 20th century. Each new drug class that emerged gave pharmacologists a new challenge, and it was then that pharmacology really established its identity and its status among the biomedical sciences.

In parallel with the exuberant proliferation of therapeutic molecules – driven mainly by chemistry – which gave pharmacologists so much to think about, physiology was also making rapid progress, particularly in relation to chemical mediators, which are discussed in depth throughout this book. Many hormones, neurotransmitters and inflammatory mediators were discovered in this period, and the realisation that chemical communication plays a central role in almost every regulatory mechanism that our bodies possess immediately established a large area of common ground between physiology and pharmacology, for interactions between chemical substances and living systems were exactly what pharmacologists had been preoccupied with from the outset. Indeed, these fields have developed hand-in-hand as wherever there is either a physiological or pathological mechanism, pharmacology could be there to exploit it with a drug. The concept of 'receptors' for chemical mediators, first proposed by Langley in 1905, was quickly taken up by pharmacologists such as Clark, Gaddum, Schild and others, and is a constant theme in present-day pharmacology (as you will soon discover as you plough through the next two chapters). The receptor concept, and the technologies developed from it, have had a massive impact on drug discovery and therapeutics. Biochemistry also emerged as a distinct science early in the 20th century, and the discovery of enzymes and the delineation of biochemical pathways provided yet another framework for understanding drug effects. The picture of pharmacology that emerges from this brief glance at history (Fig. 1.1) is of a subject evolved from ancient prescientific therapeutics, involved in commerce from the 17th century onwards, and which gained respectability by donning the trappings of science as soon as this became possible in the mid-19th century. Pharmacology grew rapidly in partnership with the evolution of organic chemistry and other biomedical sciences, and was quick to assimilate the dramatic advances in molecular and cell biology in the late 20th century. Signs of its carpetbagger past still cling to pharmacology, for the pharmaceutical industry has become very big business and much pharmacological research nowadays takes place in a commercial environment, a rougher and more pragmatic place than academia.[5] No other biomedical 'ology' is so close to Mammon.

ALTERNATIVE THERAPEUTIC PRINCIPLES

Modern medicine relies heavily on drugs as the main tool of therapeutics. Other therapeutic procedures, such

[4] A handful of synthetic substances achieved pharmacological prominence long before the era of synthetic chemistry began. Diethyl ether, first prepared as 'sweet oil of vitriol' in the 16th century, and nitrous oxide, prepared by Humphrey Davy in 1799, were used to liven up parties before being introduced as anaesthetic agents in the mid-19th century (see Ch. 42). Amyl nitrite (see Ch. 21) was made in 1859 and can claim to be the first 'rational' therapeutic drug; its therapeutic effect in angina was predicted on the basis of its physiological effects – a true 'pharmacologist's drug' and the smelly forerunner of the nitrovasodilators that are widely used today. Aspirin (Ch. 27), the most widely used therapeutic drug in history, was first synthesised in 1853, with no therapeutic application in mind. It was rediscovered in 1897 in the laboratories of the German company Bayer, who were seeking a less toxic derivative of salicylic acid. Bayer commercialised aspirin in 1899 and made a fortune.

[5] Some of our most distinguished pharmacological pioneers made their careers in industry: for example, Henry Dale, who laid the foundations of our knowledge of chemical transmission and the autonomic nervous system (Ch. 13); George Hitchings and Gertrude Elion, who described the antimetabolite principle and produced the first effective anticancer drugs (Ch. 57); and James Black, who introduced the first β-adrenoceptor and histamine H_2-receptor antagonists (Chs 15 and 31). It is no accident that in this book, where we focus on the scientific principles of pharmacology, most of our examples are products of industry, not of nature.

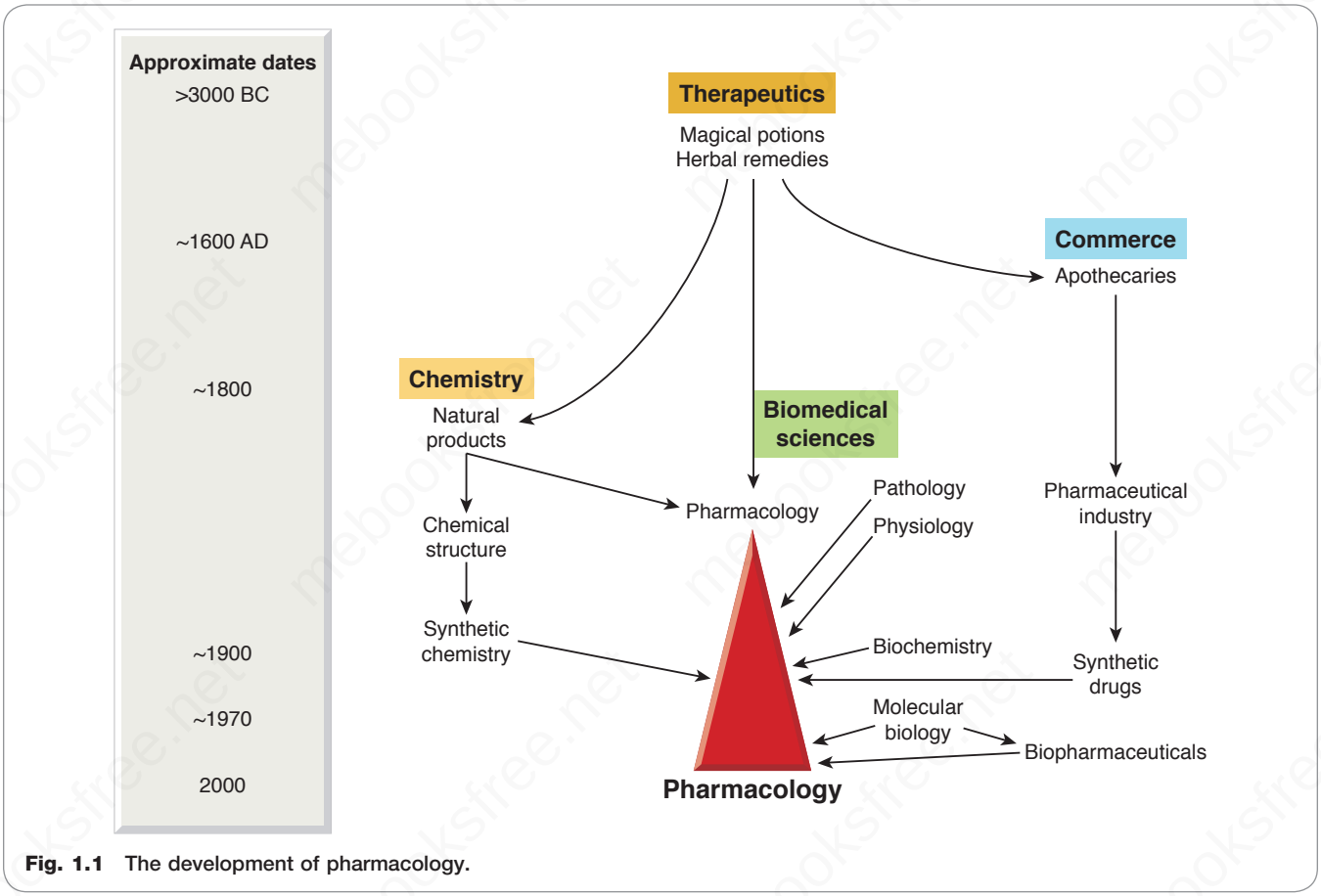

Fig. 1.1 The development of pharmacology.

as surgery, diet, exercise, psychological treatments etc., are also important, of course, as is deliberate non-intervention, but none is so widely applied as drug-based therapeutics.

Before the advent of science-based approaches, repeated attempts were made to construct systems of therapeutics, many of which produced even worse results than pure empiricism. One of these was *allopathy*, espoused by James Gregory (1735–1821). The favoured remedies included bloodletting, emetics and purgatives, which were used until the dominant symptoms of the disease were suppressed. Many patients died from such treatment, and it was in reaction against it that Hahnemann introduced the practice of *homeopathy* in the early 19th century. The implausible guiding principles of homeopathy are:

- like cures like
- activity can be enhanced by dilution

The system rapidly drifted into absurdity: for example, Hahnemann recommended the use of drugs at dilutions of $1:10^{60}$, equivalent to one molecule in a sphere the size of the orbit of Neptune.

Many other systems of therapeutics have come and gone, and the variety of dogmatic principles that they embodied have tended to hinder rather than advance scientific progress. Currently, therapeutic systems that have a basis that lies outside the domain of science remain popular under the general banner of 'alternative' or 'complementary' medicine. Mostly, they reject the 'medical model', which attributes disease to an underlying derangement of normal function that can be defined in physiological or structural terms, detected by objective means, and influenced beneficially by appropriate chemical or physical interventions. They focus instead mainly on subjective malaise, which may be disease-associated or not. Abandoning objectivity in defining and measuring disease goes along with a similar departure from scientific principles in assessing therapeutic efficacy and risk, with the result that principles and practices can gain acceptance without satisfying any of the criteria of validity that would convince a critical scientist, and that are required by law to be satisfied before a new drug can be introduced into therapy. Demand for 'alternative' therapies by the general public, alas, has little to do with demonstrable efficacy.[6]

THE EMERGENCE OF BIOTECHNOLOGY

Since the 1980s, biotechnology has emerged as a major source of new therapeutic agents in the form of antibodies, enzymes and various regulatory proteins, including hormones, growth factors and cytokines (see Clark & Pazdernik, 2015). Although such products (known as *biopharmaceuticals*, *biologicals* or *biologics*) are generally produced by genetic engineering rather than by synthetic chemistry, the pharmacological principles are essentially the same as for conventional drugs, although the details of absorption,

[6]The UK Medicines and Healthcare Regulatory Agency (MHRA) requires detailed evidence of therapeutic efficacy based on controlled clinical trials before a new drug is registered, but no clinical trials data for homeopathic products or for the many herbal medicines that were on sale before the Medicines Act of 1968.

distribution and elimination, specificity, harmful effects and clinical effectiveness all differ markedly between high molecular-weight biopharmaceuticals and low molecular-weight drugs – as does their cost! Looking further ahead, gene- and cell-based therapies (Ch. 5), although still in their infancy, are beginning to take therapeutics into a new domain. The principles governing gene suppression, the design, delivery and control of functioning artificial genes introduced into cells, or of engineered cells introduced into the body, are very different from those of drug-based therapeutics and will require a different conceptual framework, which texts such as this will increasingly need to embrace if they are to stay abreast of modern medical treatment.

PHARMACOLOGY TODAY

As with other biomedical disciplines, the boundaries of pharmacology are not sharply defined, nor are they constant. Its exponents are, as befits pragmatists, ever ready to poach on the territory and techniques of other disciplines. If it ever had a conceptual and technical core that it could really call its own, this has now dwindled almost to the point of extinction, and the subject is defined by its purpose – to understand what drugs do to living organisms, and more particularly how their effects can be applied to therapeutics – rather than by its scientific coherence.

Fig. 1.2 shows the structure of pharmacology as it appears today. Within the main subject fall a number of compartments (neuropharmacology, immunopharmacology, pharmacokinetics, etc.), which are convenient, if not watertight, subdivisions. These topics form the main subject matter of this book. Around the edges are several interface disciplines, not covered in this book, which form important two-way bridges between pharmacology and other fields of biomedicine. Pharmacology tends to have more of these than other disciplines. Recent arrivals on the fringe are subjects such as pharmacogenomics, pharmacoepidemiology and pharmacoeconomics.

Pharmacogenomics. Pharmacogenetics, the study of genetic influences on responses to drugs, initially focused on familial idiosyncratic drug reactions, where affected individuals show an abnormal – usually adverse – response to a class of drug (see Nebert & Weber, 1990). Rebranded as pharmacogenomics, it now covers broader genetically based variations in drug response, where the genetic basis is more complex, the aim being to use genetic information to guide the choice of drug therapy on an individual basis – so-called personalised medicine (Ch. 12). The underlying principle is that differences between individuals in their response to therapeutic drugs can be predicted from their genetic make-up. Examples that confirm this are steadily accumulating (see Ch. 12). So far, they mainly involve genetic polymorphism of drug-metabolising enzymes or receptors. Ultimately, linking specific gene variations with variations in therapeutic or unwanted effects of a particular drug should enable the tailoring of therapeutic choices on the basis of an individual's genotype. Steady improvements in the cost and feasibility of individual genotyping will

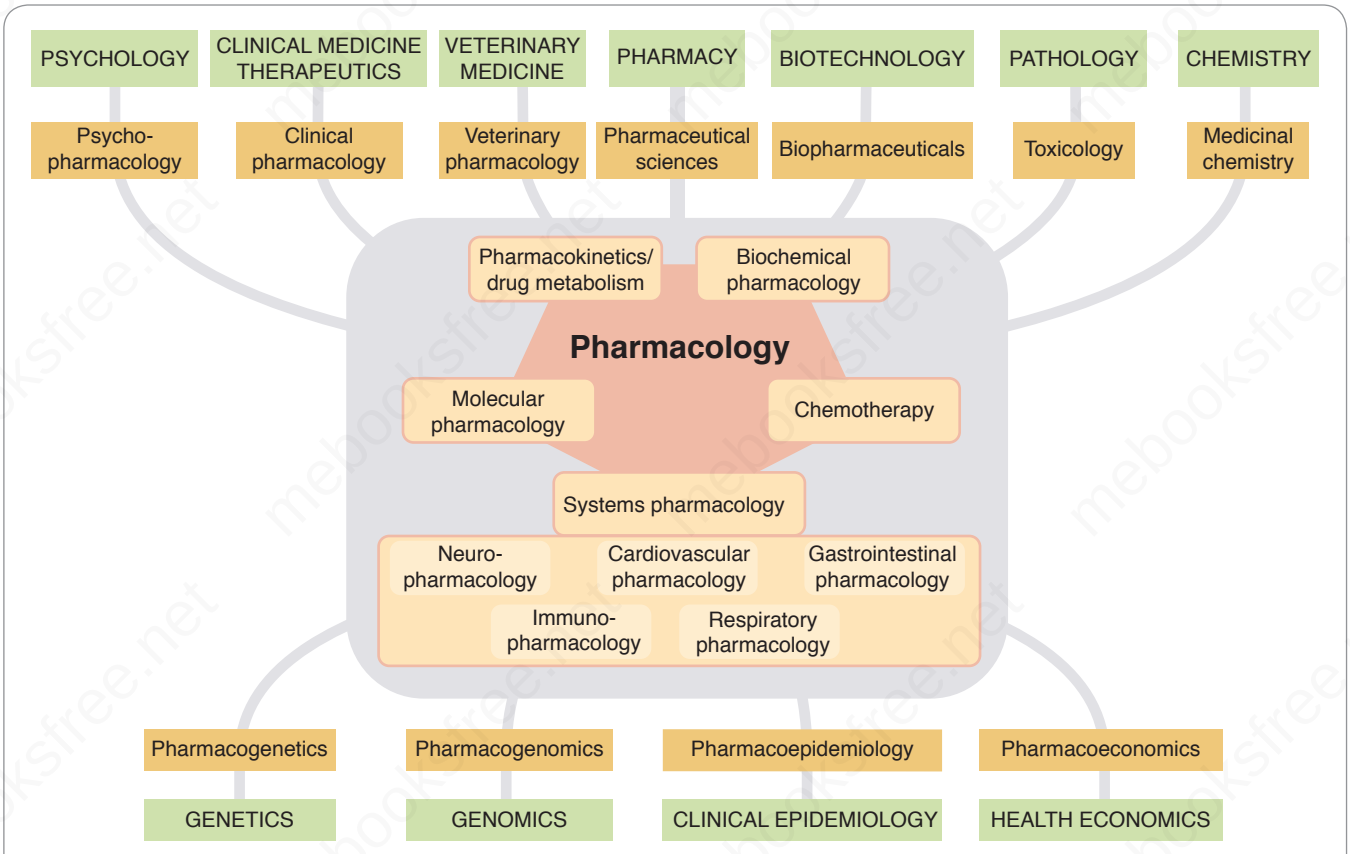

Fig. 1.2 Pharmacology today with its various subdivisions. The grey box contains the general areas of pharmacology covered in this book. Interface disciplines *(brown boxes)* link pharmacology to other mainstream biomedical disciplines *(green boxes)*.

increase its applicability, potentially with far-reaching consequences for therapeutics (see Ch. 12).

Pharmacoepidemiology. This is the study of drug effects at the population level (see Strom et al., 2013). It is concerned with the variability of drug effects between individuals in a population, and between populations. It is an increasingly important topic in the eyes of the regulatory authorities who decide whether or not new drugs can be licensed for therapeutic use. Variability between individuals or populations detracts from the utility of a drug, even though its overall effect level may be satisfactory. Pharmacoepidemiological studies also take into account patient compliance and other factors that apply when the drug is used under real-life conditions.

Pharmacoeconomics. This branch of health economics aims to quantify in economic terms the cost and benefit of drugs used therapeutically. It arose from the concern of many governments to provide for healthcare from tax revenues, raising questions of what therapeutic procedures represent the best value for money. This, of course, raises fierce controversy, because it ultimately comes down to putting monetary value on health and longevity. As with pharmacoepidemiology, regulatory authorities are increasingly requiring economic analysis, as well as evidence of individual benefit, when making decisions on licensing. For more information on this complex subject, see Rascati (2013).

REFERENCES AND FURTHER READING

Clark, D.P., Pazderink, N.J., 2015. Biotechnology. Elsevier, New York. (*General account of biotechnology and its potential applications*)

Nebert, D.W., Weber, W.W., 1990. Pharmacogenetics. In: Pratt, W.B., Taylor, P. (Eds.), Principles of Drug Action, third ed. Churchill Livingstone, New York. (*A detailed account of genetic factors that affect responses to drugs, with many examples from the pregenomic literature*)

Porter, R., 1997. The Greatest Benefit to Mankind. Harper-Collins, London. (*An excellent and readable account of the history of medicine, with good coverage of the early development of pharmacology and the pharmaceutical industry*)

Rascati, K.L., 2013. Essentials of Pharmacoeconomics, second ed. Lippincott Williams & Wilkins, Philadelphia. (*Introduction to a complex and fraught subject*)

Strom, B.L., Kimmel, S.E., Hennessy, S., 2013. Textbook of Pharmacoepidemiology, second ed. Wiley, Chichester. (*A multiauthor book covering all aspects of a newly emerged discipline, including aspects of pharmacoeconomics*)

2 How drugs act: general principles

OVERVIEW

The emergence of pharmacology as a science came when the emphasis shifted from describing what drugs do to explaining how they work. In this chapter we set out some general principles underlying the interaction of drugs with living systems (Ch. 3 goes into the molecular aspects in more detail). The interaction between drugs and cells is described, followed by a more detailed examination of different types of drug–receptor interaction. The receptor concept has been described as the 'big idea' of pharmacology (Rang, 2006) and will be a recurring theme throughout this book.

INTRODUCTION

To begin with, we should gratefully acknowledge Paul Ehrlich for insisting that drug action must be explicable in terms of conventional chemical interactions between drugs and tissues, and for dispelling the idea that the remarkable potency and specificity of action of some drugs put them somehow out of reach of chemistry and physics and required the intervention of magical 'vital forces'. Although many drugs produce effects in extraordinarily low doses and concentrations, low concentrations still involve very large numbers of molecules. One drop of a solution of a drug at only 10^{-10} mol/L still contains about 3×10^9 drug molecules, so there is no mystery in the fact that it may produce an obvious pharmacological response. Some bacterial toxins (e.g. diphtheria toxin) act with such precision that a single molecule taken up by a target cell is sufficient to kill it.

One of the basic tenets of pharmacology is that drug molecules must exert some chemical influence on one or more cell constituents in order to produce a pharmacological response. In other words, drug molecules must get so close to these constituent cellular molecules that the two interact chemically in such a way that the function of the latter is altered. Of course, the molecules in the organism vastly outnumber the drug molecules, and if the drug molecules were merely distributed at random, the chance of interaction with any particular class of cellular molecule would be negligible. Therefore pharmacological effects require, in general, the non-uniform distribution of the drug molecule within the body or tissue, which is the same as saying that drug molecules must be 'bound' to particular constituents of cells and tissues in order to produce an effect. Ehrlich summed it up thus: '*Corpora non agunt nisi fixata*' (in this context, 'A drug will not work unless it is bound').[1]

These critical binding sites are often referred to as 'drug targets' (an obvious allusion to Ehrlich's famous phrase 'magic bullets', describing the potential of antimicrobial drugs). The mechanisms by which the association of a drug molecule with its target leads to a physiological response constitute the major thrust of pharmacological research. Most drug targets are protein molecules. Even general anaesthetics (see Ch. 42), which were long thought to produce their effects by an interaction with membrane lipid, now appear to interact mainly with membrane proteins (see Franks, 2008).

All rules need exceptions, and many antimicrobial and antitumour drugs (Chs 52 and 57), as well as mutagenic and carcinogenic agents (Ch. 58), interact directly with DNA rather than protein; bisphosphonates, used to treat osteoporosis (Ch. 37), bind to calcium salts in the bone matrix, rendering them toxic to osteoclasts, much like rat poison. There are also exceptions among the new generation of *biopharmaceutical drugs* that include nucleic acids, proteins and antibodies (see Ch. 5).

PROTEIN TARGETS FOR DRUG BINDING

Four main kinds of regulatory protein are commonly involved as primary drug targets, namely:

- receptors
- enzymes
- carrier molecules (transporters)
- ion channels

Furthermore, many drugs bind (in addition to their primary targets) to plasma proteins (see Ch. 9) and other tissue proteins, without producing any obvious physiological effect. Nevertheless, the generalisation that most drugs act on one or other of the four types of protein listed above serves as a good starting point.

Further discussion of the mechanisms by which such binding leads to cellular responses is given in Chapters 3–4.

DRUG RECEPTORS

WHAT DO WE MEAN BY RECEPTORS?

▼ As emphasised in Chapter 1, the concept of receptors is central to pharmacology, and the term is most often used to describe the target molecules through which soluble physiological mediators – hormones, neurotransmitters, inflammatory mediators, etc. – produce their effects. Examples such as acetylcholine receptors, cytokine receptors, steroid receptors and growth hormone receptors abound in this book, and generally the term *receptor* indicates a recognition molecule for a chemical mediator through which a response is transduced.

'Receptor' is sometimes used to denote *any* target molecule with which a drug molecule (i.e. a foreign compound rather than an endogenous mediator) has to combine in order to elicit its specific

[1]There are, if one looks hard enough, exceptions to Ehrlich's dictum – drugs that act without being bound to any tissue constituent (e.g. osmotic diuretics, osmotic purgatives, antacids and heavy metal chelating agents). Nonetheless, the principle remains true for the great majority.

Targets for drug action

- A drug is a chemical applied to a physiological system that affects its function in a specific way.
- With some exceptions, drugs act on target proteins, namely:
  - receptors
  - enzymes
  - carriers
  - ion channels.
- The term *receptor* is used in different ways. In pharmacology, it describes protein molecules whose function is to recognise and respond to endogenous chemical signals. Other macromolecules with which drugs interact to produce their effects are known as *drug targets*.
- Specificity is reciprocal: individual classes of drug bind only to certain targets, and individual targets recognise only certain classes of drug.
- No drugs are completely specific in their actions. In many cases, increasing the dose of a drug will cause it to affect targets other than the principal one, and this can lead to side effects.

effect. For example, the voltage-sensitive sodium channel is sometimes referred to as the 'receptor' for **local anaesthetics** (see Ch. 44), or the enzyme dihydrofolate reductase as the 'receptor' for **methotrexate** (Ch. 51). The term *drug target*, of which receptors are one type, is preferable in this context.

In the more general context of cell biology, the term receptor is used to describe various cell surface molecules (such as *T-cell receptors*, *integrins*, *Toll receptors*, etc; see Ch. 7) involved in the cell-to-cell interactions that are important in immunology, cell growth, migration and differentiation, some of which are also emerging as drug targets. These receptors differ from conventional pharmacological receptors in that they respond to proteins attached to cell surfaces or extracellular structures, rather than to soluble mediators.

Various carrier proteins are often referred to as receptors, such as the *low-density lipoprotein receptor* that plays a key role in lipid metabolism (Ch. 24) and the *transferrin receptor* involved in iron absorption (Ch. 26). These entities have little in common with pharmacological receptors. Though quite distinct from pharmacological receptors, these proteins play an important role in the action of drugs such as *statins* (Ch. 24).

RECEPTORS IN PHYSIOLOGICAL SYSTEMS

Receptors form a key part of the system of chemical communication that all multicellular organisms use to coordinate the activities of their cells and organs. Without them, we would be unable to function.

Some fundamental properties of receptors are illustrated by the action of **adrenaline** (epinephrine) on the heart. Adrenaline first binds to a receptor protein (the β_1 *adrenoceptor*, see Ch. 15) that serves as a recognition site for adrenaline and other catecholamines. When it binds to the receptor, a train of reactions is initiated (see Ch. 3), leading to an increase in force and rate of the heartbeat. In the absence of adrenaline, the receptor is normally functionally silent. This is true of most receptors for endogenous mediators (hormones, neurotransmitters, cytokines, etc.), although there are examples (see Ch. 3) of receptors that are 'constitutively active' – that is, they exert a controlling influence even when no chemical mediator is present (see p. 14).

There is an important distinction between *agonists*, which 'activate' the receptors, and *antagonists*, which combine at the same site without causing activation, and block the effect of agonists on that receptor. The distinction between agonists and antagonists only exists for pharmacological receptors; we cannot usefully speak of 'agonists' for the other classes of drug target described above.

The characteristics and accepted nomenclature of pharmacological receptors are described by Neubig et al. (2003). The origins of the receptor concept and its pharmacological significance are discussed by Rang (2006).

DRUG SPECIFICITY

For a drug to be useful as either a therapeutic or a scientific tool, it must act selectively on particular cells and tissues. In other words, it must show a high degree of binding site specificity. Conversely, proteins that function as drug targets generally show a high degree of ligand specificity; they bind only molecules of a certain precise type.

These principles of binding site and ligand specificity can be clearly recognised in the actions of a mediator such as **angiotensin** (Ch. 23). This peptide acts strongly on vascular smooth muscle, and on the kidney tubule, but has very little effect on other kinds of smooth muscle or on the intestinal epithelium. Other mediators affect a quite different spectrum of cells and tissues, the pattern in each case reflecting the specific pattern of expression of the protein receptors for the various mediators. A small chemical change, such as conversion of one of the amino acids in angiotensin from L to D form, or removal of one amino acid from the chain, can inactivate the molecule altogether, because the receptor fails to bind the altered form. The complementary specificity of ligands and binding sites, which gives rise to the very exact molecular recognition properties of proteins, is central to explaining many of the phenomena of pharmacology. It is no exaggeration to say that the ability of proteins to interact in a highly selective way with other molecules – including other proteins – is the basis of living machines. Its relevance to the understanding of drug action will be a recurring theme in this book.

Finally, it must be emphasised that no drug acts with complete specificity. Thus tricyclic antidepressant drugs (Ch. 48) act by blocking monoamine transporters but are notorious for producing side effects (e.g. dry mouth) related to their ability to block various other receptors. In general, the lower the potency of a drug and the higher the dose needed, the more likely it is that sites of action other than the primary one will assume significance. In clinical terms, this is often associated with the appearance of unwanted 'off-target' side effects,[2] of which no drug is free.

Since the 1970s, pharmacological research has succeeded in identifying the protein targets of many different types of drug. Drugs such as opioid analgesics (Ch. 43), cannabinoids (Ch. 20) and benzodiazepine tranquillisers (Ch. 45), whose actions had been described in exhaustive detail for many years, are now known to target well-defined receptors, many of which have been fully characterised by

[2]'On-target' side effects are unwanted effects mediated through the same receptor as the clinically desired effect, for example constipation and respiratory depression by opioid analgesic drugs (see Ch. 43), whereas 'off target' side effects are mediated by a different mechanism.

gene-cloning and protein crystallography techniques (see Ch. 3).

RECEPTOR CLASSIFICATION

▼ Where the action of a drug can be associated with a particular receptor, this provides a valuable means for classification and refinement in drug design. For example, pharmacological analysis of the actions of histamine (see Ch. 18) showed that some of its effects (the H_1 effects, such as smooth muscle contraction) were strongly antagonised by the competitive histamine antagonists then known. Black and his colleagues suggested in 1970 that the remaining actions of histamine, which included its stimulant effect on gastric secretion, might represent a second class of histamine receptor (H_2). Testing a number of histamine analogues, they found that some were selective in producing H_2 effects, with little H_1 activity. By analysing which parts of the histamine molecule conferred this type of specificity, they were able to develop selective H_2 antagonists, which proved to be potent in blocking gastric acid secretion, a development of major therapeutic significance (Ch. 31).[3] Two further types of histamine receptor (H_3 and H_4) were recognised later.

Receptor classification based on pharmacological responses continues to be a valuable and widely used approach. Subsequently, newer experimental approaches produced other criteria on which to base receptor classification. The direct measurement of ligand binding to receptors (see later) allowed many new receptor subtypes to be defined that could not easily be distinguished by studies of drug effects. Molecular sequencing of the amino acid structure (see Ch. 3) provided a completely new basis for classification at a much finer level of detail than can be reached through pharmacological analysis. Finally, analysis of the biochemical pathways that are linked to receptor activation (see Ch. 3) provides yet another basis for classification.

The result of this data explosion was that receptor classification suddenly became much more detailed, with a proliferation of receptor subtypes for all the main types of ligand. As alternative molecular and biochemical classifications began to spring up that were incompatible with the accepted pharmacologically defined receptor classes, the International Union of Basic and Clinical Pharmacology (IUPHAR) convened expert working groups to produce agreed receptor classifications for the major types, taking into account the pharmacological, molecular and biochemical information available. These wise people have a hard task; their conclusions will be neither perfect nor final but are essential to ensure a consistent terminology. To the student, this may seem an arcane exercise in taxonomy, generating much detail but little illumination. There is a danger that the tedious lists of drug names, actions and side effects that used to burden the subject will be replaced by exhaustive tables of receptors, ligands and transduction pathways. In this book, we have tried to avoid detail for its own sake and include only such information on receptor classification as seems interesting in its own right or is helpful in explaining the actions of important drugs. A comprehensive database of known receptor classes is available (see <www.guidetopharmacology.org/>), as well as a regularly updated summary (Alexander et al., 2015).

DRUG–RECEPTOR INTERACTIONS

Occupation of a receptor by a drug molecule may or may not result in *activation* of the receptor. By activation, we mean that the receptor is affected by the bound molecule in such a way as to alter the function of the cell and elicit a tissue response. The molecular mechanisms associated with receptor activation are discussed in Chapter 3. Binding and activation represent two distinct steps in the generation of the receptor-mediated response by an agonist (Fig. 2.1). If a drug binds to the receptor without causing activation and thereby prevents the agonist from binding, it is termed a *receptor antagonist*. The tendency of a drug to bind to the receptors is governed by its *affinity*, whereas the tendency for it, once bound, to activate the receptor is denoted by its *efficacy*. These terms are defined more precisely later (pp. 9 and 11). Drugs of high potency generally have a high affinity for the receptors and thus occupy a significant proportion of the receptors even at low concentrations. Agonists also possess significant efficacy, whereas antagonists, in the simplest case, have zero efficacy. Drugs with intermediate levels of efficacy, such that even when 100% of the receptors are occupied the tissue response is submaximal, are known as *partial agonists*, to distinguish them from *full agonists*, the efficacy of which is sufficient that they can elicit a maximal tissue response. These concepts, though clearly an oversimplified description of events at the molecular level (see Ch. 3), provide a useful basis for characterising drug effects.

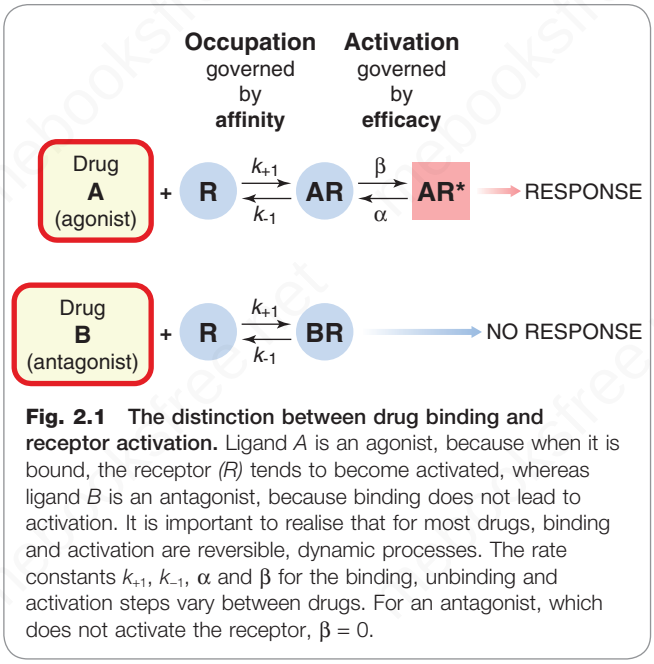

Fig. 2.1 The distinction between drug binding and receptor activation. Ligand *A* is an agonist, because when it is bound, the receptor *(R)* tends to become activated, whereas ligand *B* is an antagonist, because binding does not lead to activation. It is important to realise that for most drugs, binding and activation are reversible, dynamic processes. The rate constants k_{+1}, k_{-1}, α and β for the binding, unbinding and activation steps vary between drugs. For an antagonist, which does not activate the receptor, β = 0.

We now discuss certain aspects in more detail, namely drug binding, agonist concentration–effect curves, competitive antagonism, partial agonists and the nature of efficacy. Understanding these concepts at a qualitative level is sufficient for many purposes, but for more detailed analysis a quantitative formulation is needed (see pp. 19–20).

THE BINDING OF DRUGS TO RECEPTORS

▼ The binding of drugs to receptors can often be measured directly by the use of drug molecules (agonists or antagonists) labelled with one or more radioactive atoms (usually ^{3}H, ^{14}C or ^{125}I). The usual procedure is to incubate samples of the tissue (or membrane fragments) with various concentrations of radioactive drug until equilibrium is reached (i.e. when the rate of association [binding] and dissociation [unbinding] of the radioactive drug are equal). The bound radioactivity is measured after removal of the supernatant.

In such experiments, the radiolabelled drug will exhibit both specific binding (i.e. binding to receptors, which is saturable as there are a finite number of receptors in the tissue) and a certain amount of 'non-specific binding' (i.e. drug taken up by structures other than receptors, which, at the concentrations used in such studies, is normally non-saturable), which obscures the specific component and needs to be kept to a minimum (Fig. 2.2A–B). The amount of non-specific

[3]For this work, and the development of β-adrenoceptor antagonists by a similar experimental approach, Sir James Black was awarded the 1984 Nobel Prize in Physiology or Medicine.

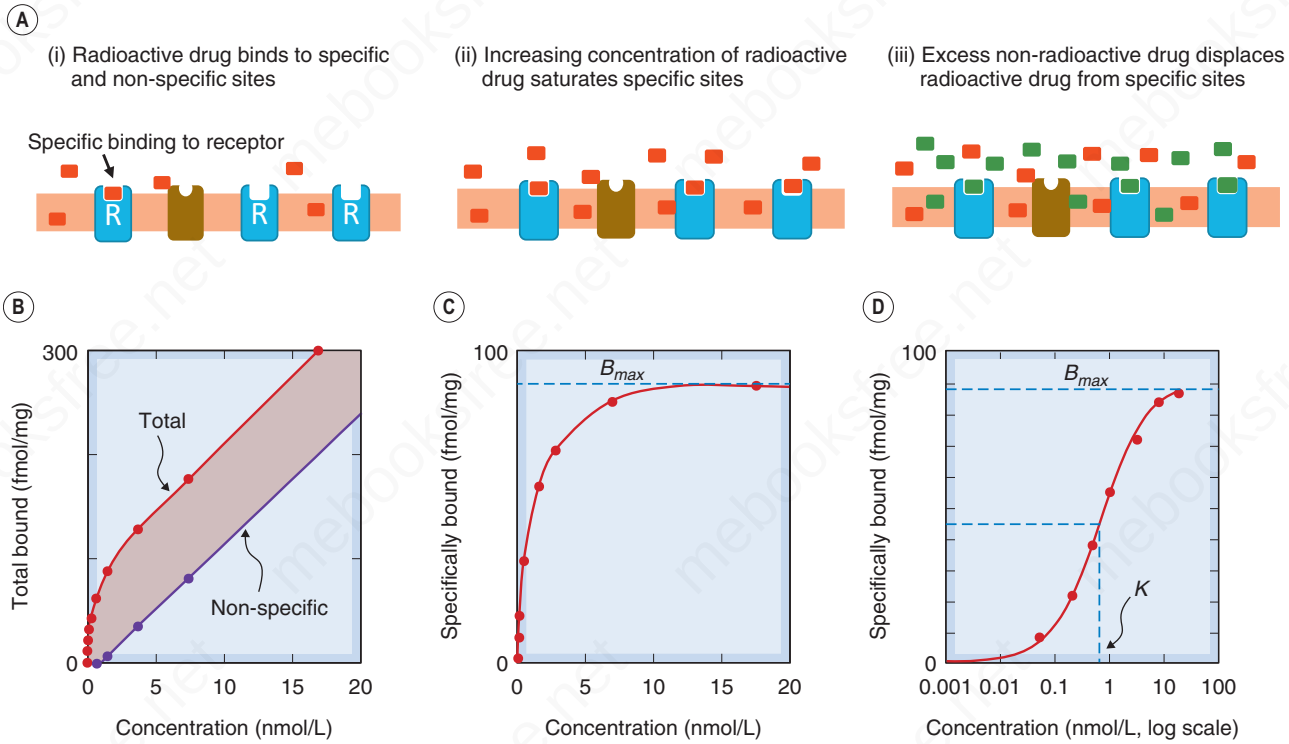

Fig. 2.2 Measurement of receptor binding. (A) (i) Cartoon depicting radioligand (shown in red) binding to its receptor *(R)* in the membrane as well as to non-specific sites on other proteins and lipid. In (ii) when the concentration of radioligand is increased all the specific sites become saturated but non-specific binding continues to increase. In (iii) addition of a high concentration of a non-radioactive drug *(shown in green)* that also binds to R displaces the radioactive drug from its receptors but not from the non-specific sites. (B–D) Illustrate actual experimental results for radioligand binding to β adrenoceptors in cardiac cell membranes. The ligand was [^{3}H]-cyanopindolol, a derivative of pindolol (see Ch. 15). (B) Measurements of total and non-specific binding at equilibrium. Non-specific binding is measured in the presence of a saturating concentration of a non-radioactive β-adrenoceptor agonist, which prevents the radioactive ligand from binding to β adrenoceptors. The difference between the two lines represents specific binding. (C) Specific binding plotted against concentration. The curve is a rectangular hyperbola (Eq. 2.5). (D) Specific binding as in (C) plotted against the concentration on a log scale. The sigmoid curve is a *logistic curve* representing the logarithmic scaling of the rectangular hyperbola plotted in panel (C) from which the binding parameters K (the equilibrium dissociation constant) and B_{max} (the binding capacity) can be determined.

binding is estimated by measuring the radioactivity taken up in the presence of a saturating concentration of a (non-radioactive) ligand that inhibits completely the binding of the radioactive drug to the receptors, leaving behind the non-specific component. This is then subtracted from the total binding to give an estimate of specific binding (Fig. 2.2C). The *binding curve* (Fig. 2.2C–D) defines the relationship between concentration and the amount of drug bound (B), and in most cases it fits well to the relationship predicted theoretically (see Fig. 2.14), allowing the affinity of the drug for the receptors to be estimated, as well as the *binding capacity* (B_{max}), representing the density of receptors in the tissue. When combined with functional studies, binding measurements have proved very valuable. It has, for example, been confirmed that the *spare receptor hypothesis* (p. 10) for muscarinic receptors in smooth muscle is correct; agonists are found to bind, in general, with rather low affinity, and a maximal biological effect occurs at low receptor occupancy. It has also been shown, in skeletal muscle and other tissues, that denervation leads to an increase in the number of receptors in the target cell, a finding that accounts, at least in part, for the phenomenon of *denervation supersensitivity*. More generally, it appears that receptors tend to increase in number, usually over the course of a few days, if the relevant hormone or transmitter is absent or scarce, and to decrease in number if the receptors are activated for a prolonged period, a process of adaptation to continued administration of drugs or hormones (see p. 18).

Non-invasive imaging techniques, such as *positron emission tomography* (PET), using drugs labelled with an isotope of short half-life (such as ^{11}C or ^{18}Fl), can also be used to investigate the distribution of receptors in structures such as the living human brain. This technique has been used, for example, to measure the degree of dopamine-receptor blockade produced by antipsychotic drugs in the brains of schizophrenic patients (see Ch. 47).

Binding curves with agonists often reveal an apparent heterogeneity among receptors. For example, agonist binding to muscarinic receptors (Ch. 14) and also to β adrenoceptors (Ch. 15) suggests at least two populations of binding sites with different affinities. This may be because the receptors can exist either unattached or coupled within the membrane to another macromolecule, the G protein (see Ch. 3), which constitutes part of the transduction system through which the receptor exerts its regulatory effect. Antagonist binding does not show this complexity, probably because antagonists, by their nature, do not lead to the secondary event of G protein coupling. Because agonist binding results in activation, agonist affinity has proved to be a surprisingly elusive concept, about which aficionados love to argue.

THE RELATION BETWEEN DRUG CONCENTRATION AND EFFECT

Although binding can be measured directly, it is usually a biological response, such as a rise in blood pressure,

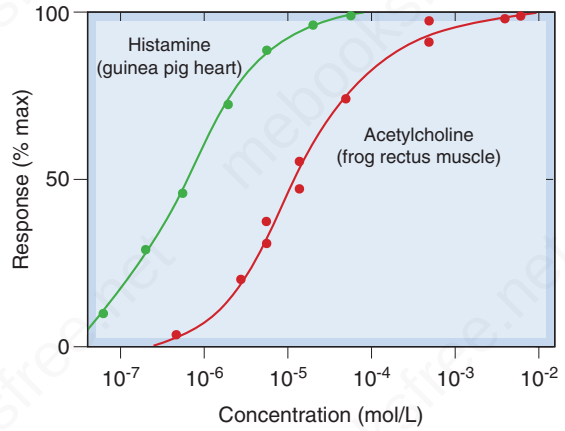

Fig. 2.3 Experimentally observed concentration–effect curves. Although the lines, drawn according to the binding Eq. 2.5, fit the points well, such curves do not give correct estimates of the affinity of drugs for receptors. This is because the relationship between receptor occupancy and response is usually non-linear.

contraction or relaxation of a strip of smooth muscle in an organ bath, the activation of an enzyme, or a behavioural response, that we are interested in, and this is often plotted as a *concentration–effect curve* (in vitro) or *dose–response curve* (in vivo), as in Fig. 2.3. This allows us to estimate the *maximal response* that the drug can produce (E_{max}), and the concentration or dose needed to produce a 50% maximal response (EC_{50} or ED_{50}). A logarithmic concentration or dose scale is often used. This transforms the curve from a rectangular hyperbola to a sigmoidal curve in which the mid portion is essentially linear (the importance of the slope of the linear portion will become apparent later in this chapter when we consider antagonism and partial agonists). The E_{max}, EC_{50} and slope parameters are useful for comparing different drugs that produce qualitatively similar effects (see Fig. 2.7 and Ch. 8). Although they look similar to the binding curve in Fig. 2.2D, concentration–effect curves cannot be used to measure the affinity of agonist drugs for their receptors, because the response produced is not, as a rule, directly proportional to receptor occupancy. This often arises because the maximum response of a tissue may be produced by agonists when they occupy less than 100% of the receptors. Under these circumstances the tissue is said to possess spare receptors (see later).

In interpreting concentration–effect curves, it must be remembered that the concentration of the drug at the receptors may differ from the known concentration in the bathing solution. Agonists may be subject to rapid enzymic degradation or uptake by cells as they diffuse from the surface towards their site of action, and a steady state can be reached in which the agonist concentration at the receptors is very much less than the concentration in the bath. In the case of acetylcholine, for example, which is hydrolysed by cholinesterase present in most tissues (see Ch. 14), the concentration reaching the receptors can be less than 1% of that in the bath, and an even bigger difference has been found with noradrenaline (norepinephrine), which is avidly taken up by sympathetic nerve terminals in many tissues (Ch. 15). The problem is reduced but not entirely eradicated by the use of recombinant receptors expressed in cells in culture. Thus, even if the concentration–effect curve, as in Fig. 2.3, looks just like a facsimile of the binding curve (see Fig. 2.2D), it cannot be used directly to determine the affinity of the agonist for the receptors.

SPARE RECEPTORS

▼ Stephenson (1956), studying the actions of acetylcholine analogues in isolated tissues, found that many full agonists were capable of eliciting maximal responses at very low occupancies, often less than 1%. This means that the mechanism linking the response to receptor occupancy has a substantial reserve capacity. Such systems may be said to possess *spare receptors*, or a receptor reserve. The existence of spare receptors does not imply any functional subdivision of the receptor pool, but merely that the pool is larger than the number needed to evoke a full response. This surplus of receptors over the number actually needed might seem a wasteful biological arrangement. But in fact it is highly efficient in that a given number of agonist–receptor complexes, corresponding to a given level of biological response, can be reached with a lower concentration of hormone or neurotransmitter than would be the case if fewer receptors were provided. Economy of hormone or transmitter secretion is thus achieved at the expense of providing more receptors.

COMPETITIVE ANTAGONISM

Though one drug can inhibit the response to another in several ways (see p. 16), competition at the receptor level is particularly important, both in the laboratory and in the clinic, because of the high potency and specificity that can be achieved.

In the presence of a competitive antagonist, the agonist occupancy (i.e. proportion of receptors to which the agonist is bound) at a given agonist concentration is reduced, because the receptor can accommodate only one molecule at a time. However, because the two are in competition, raising the agonist concentration can restore the agonist occupancy (and hence the tissue response). The antagonism is therefore said to be *surmountable*, in contrast to other types of antagonism (see later) where increasing the agonist concentration fails to overcome the blocking effect. A simple theoretical analysis (see p. 20) predicts that in the presence of a fixed concentration of the antagonist, the log concentration–effect curve for the agonist will be shifted to the right, without any change in slope or maximum – the hallmark of competitive antagonism (Fig. 2.4A). The shift is expressed as a *dose ratio*, *r* (the ratio by which the agonist concentration has to be increased in the presence of the antagonist in order to restore a given level of response). Theory predicts that the dose ratio increases linearly with the concentration of the antagonist (see p. 20). These predictions are often borne out in practice (Fig. 2.5A), providing a relatively simple method for determining the equilibrium dissociation constant of the antagonist (K_B; Fig. 2.5B). Examples of competitive antagonism are very common in pharmacology. The surmountability of the block by the antagonist may be important in practice, because it allows the functional effect of the agonist to be restored by an increase in concentration. With other types of antagonism (as detailed below), the block is usually insurmountable.

The salient features of competitive antagonism are:

- shift of the agonist log concentration–effect curve to the right, without change of slope or maximum (i.e. antagonism can be overcome by increasing the concentration of the agonist)

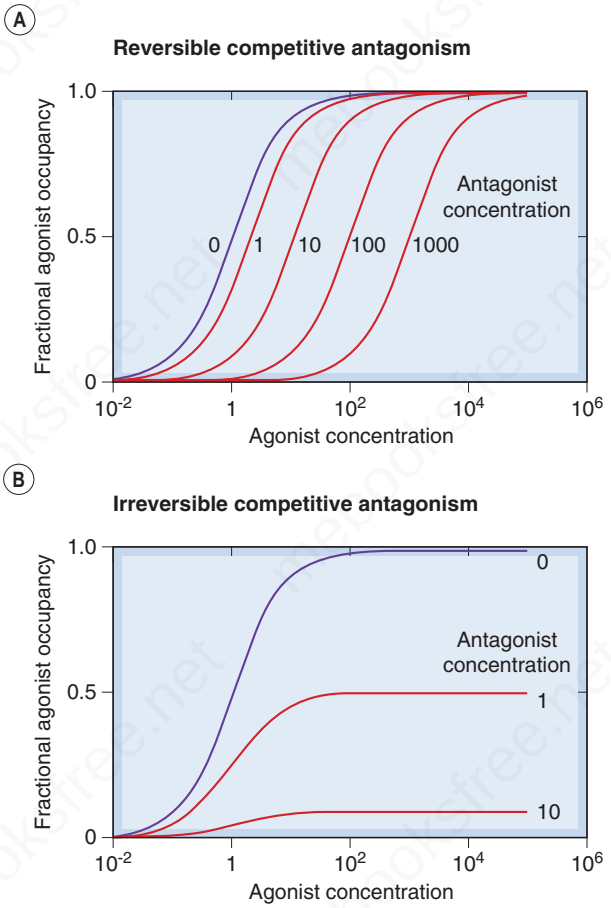

Fig. 2.4 Hypothetical agonist concentration–occupancy curves in the presence of reversible (A) and irreversible (B) competitive antagonists. The concentrations are normalised with respect to the equilibrium dissociation constants, *K* (i.e. 1.0 corresponds to a concentration equal to *K* and results in 50% occupancy). Note that in (A) increasing the agonist concentration overcomes the effect of a reversible antagonist (i.e. the block is surmountable), so that the maximal response is unchanged, whereas in (B) the effect of an irreversible antagonist is insurmountable and full agonist occupancy cannot be achieved.

- linear relationship between agonist dose ratio and antagonist concentration
- evidence of competition from binding studies.

Competitive antagonism is the most direct mechanism by which one drug can reduce the effect of another (or of an endogenous mediator).

▼ The characteristics of *reversible competitive antagonism* described above reflect the fact that agonist and competitive antagonist molecules do not stay bound to the receptor but dissociate and rebind continuously. The rate of dissociation of the antagonist molecules is sufficiently high that a new equilibrium is rapidly established on addition of the agonist. In effect, agonist molecules are able to replace the antagonist molecules on the receptors when the antagonist unbinds, although they cannot, of course, evict bound antagonist molecules. Displacement occurs because, by occupying a proportion of the vacant receptors, the agonist effectively reduces the rate of association of the antagonist molecules; consequently, the rate of dissociation temporarily exceeds that of association, and the overall antagonist occupancy falls.

Competitive antagonism

- Reversible competitive antagonism is the commonest and most important type of antagonism; it has two main characteristics.
  - In the presence of the antagonist, the agonist log concentration–effect curve is shifted to the right without change in slope or maximum, the extent of the shift being a measure of the *dose ratio.*
  - The dose ratio increases linearly with antagonist concentration.
- Antagonist affinity, measured in this way, is widely used as a basis for receptor classification.

IRREVERSIBLE COMPETITIVE ANTAGONISM

▼ *Irreversible competitive* (or *non-equilibrium*) *antagonism* occurs when the antagonist binds to the same site on the receptor as the agonist but dissociates very slowly, or not at all, from the receptors, with the result that no change in the antagonist occupancy takes place when the agonist is applied.[4]

The predicted effects of reversible and irreversible antagonists are compared in Fig. 2.4.

In some cases (Fig. 2.6A), the theoretical effect is accurately reproduced with the antagonist reducing the maximum response. However, the distinction between reversible and irreversible competitive antagonism (or even non-competitive antagonism) is not always so clear. This is because of the phenomenon of spare receptors (see p. 10); if the agonist occupancy required to produce a maximal biological response is very small (say 1% of the total receptor pool), then it is possible to block irreversibly nearly 99% of the receptors without reducing the maximal response. The effect of a lesser degree of antagonist occupancy will be to produce a parallel shift of the log concentration–effect curve that is indistinguishable from reversible competitive antagonism (Fig. 2.6B). Only when the antagonist occupancy exceeds 99% will the maximum response will be reduced.

Irreversible competitive antagonism occurs with drugs that possess reactive groups that form covalent bonds with the receptor. These are mainly used as experimental tools for investigating receptor function, and few are used clinically. Irreversible enzyme inhibitors that act similarly are clinically used, however, and include drugs such as **aspirin** (Ch. 27), **omeprazole** (Ch. 31) and monoamine oxidase inhibitors (Ch. 48).

PARTIAL AGONISTS AND THE CONCEPT OF EFFICACY

So far, we have considered drugs either as agonists, which in some way activate the receptor when they occupy it, or as antagonists, which cause no activation. However, the ability of a drug molecule to activate the receptor – namely its efficacy – is actually a graded, rather than an all-or-nothing, property. If a series of chemically related agonist drugs acting on the same receptors is tested on a given biological system, it is often found that the largest response that can be produced differs from one drug to another. Some compounds (known as *full agonists*) can produce a maximal response (the largest response that the tissue is capable of giving), whereas others (*partial agonists*) can produce only a submaximal response. Fig. 2.7A shows concentration–effect curves for several α-adrenoceptor agonists (see Ch. 15), which cause contraction of isolated

[4]This type of antagonism is sometimes called non-competitive, but that term is ambiguous and best avoided in this context.

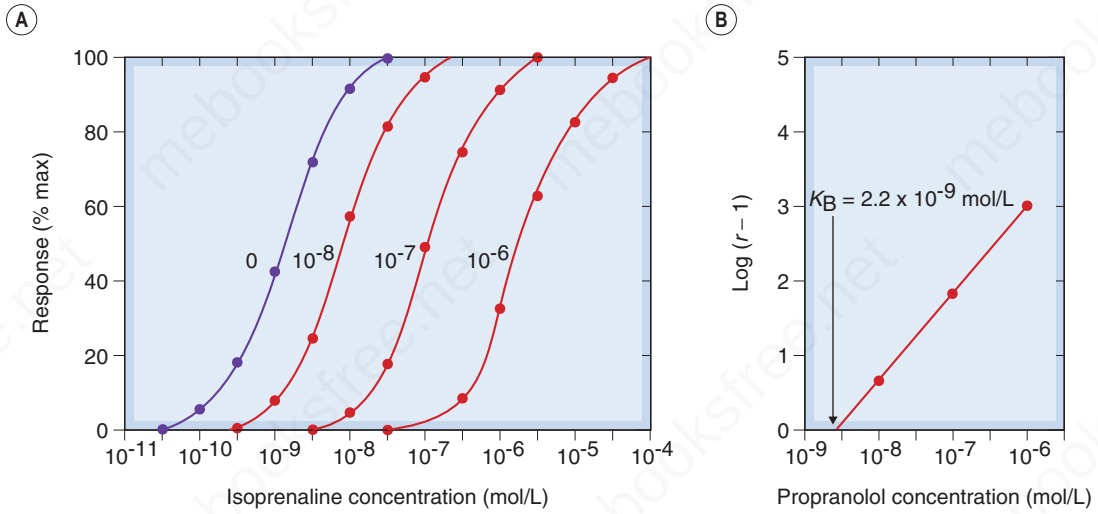

Fig. 2.5 Competitive antagonism of isoprenaline by propranolol measured on isolated guinea pig atria. (A) Concentration–effect curves at various propranolol concentrations (indicated on the curves). Note the progressive shift to the right without a change of slope or maximum. (B) Schild plot (Eq. 2.10). The equilibrium dissociation constant (K_B) for propranolol is given by the abscissal intercept, 2.2×10^{-9} mol/L. Note that the subscript 'B' is now used in 'K_B' to indicate that the equilibrium dissociation constant is that of the antagonist (designated drug B) measured in the presence of the agonist (designated drug A). (Results from Potter, L.T., 1967. Uptake of propranolol by isolated guinea pig atria. J. Pharmacol. Exp. Ther. 55, 91–100.)

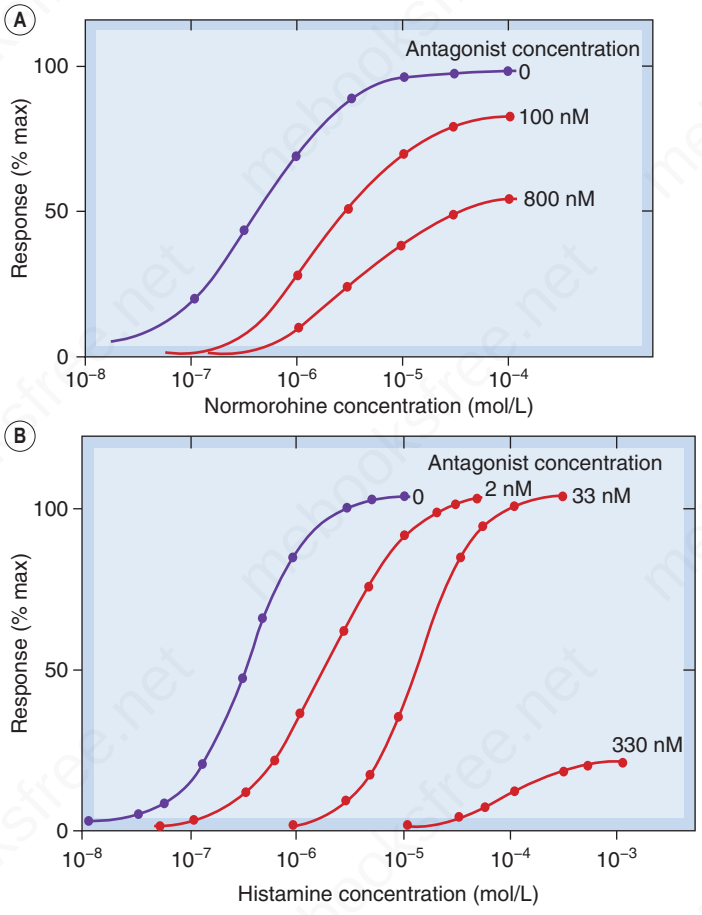

Fig. 2.6 Effects of irreversible competitive antagonists on agonist concentration–effect curves. (A) Rat brain neurones responding to the opioid agonist normorphine before and after being exposed to the irreversible competitive antagonist β-funaltrexamine for 30 minutes and then washed to remove the antagonist. Note the depression of the maximum response. (B) Responses of the guinea pig ileum to histamine before and after treatment with increasing concentrations of a receptor alkylating agent (GD121) for 5 minutes and then washed to remove the antagonist. Note the concentration–response curve is initially shifted to the right with no depression of the maximum response. (Panel [A] after Williams, J.T., North, R.A., 1984. Mol. Pharmacol. 26, 489–497; panel [B] after Nickerson, M., 1955. Nature 178, 696–697.)

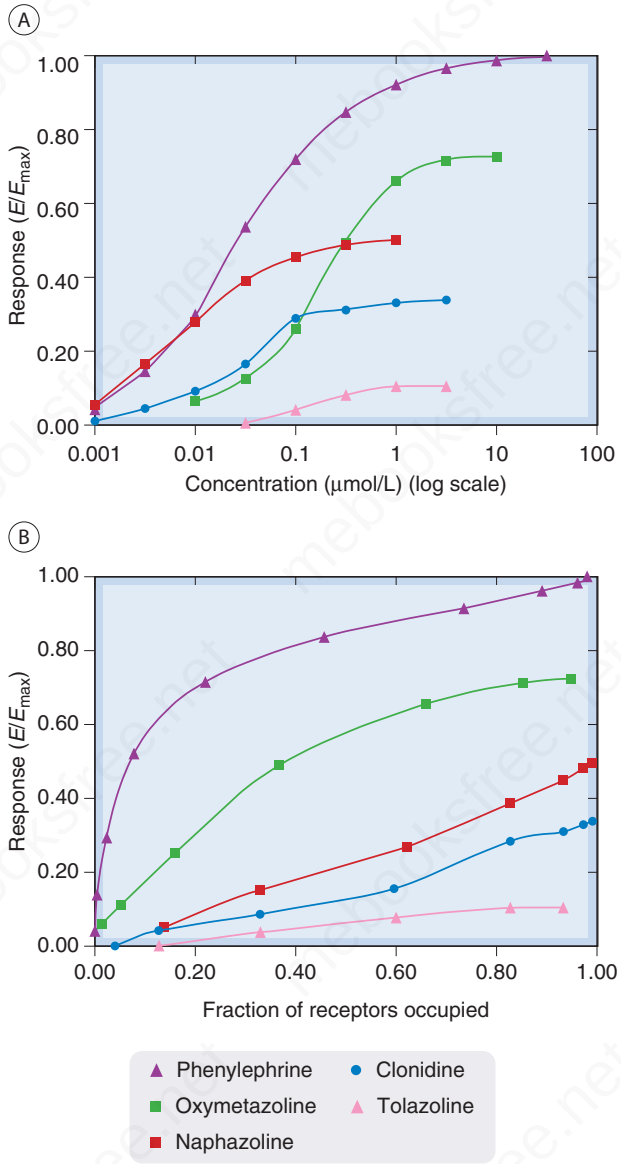

Fig. 2.7 Partial agonists. (A) Log concentration–effect curves for a series of α-adrenoceptor agonists causing contraction of an isolated strip of rabbit aorta. Phenylephrine is a full agonist. The others are partial agonists with different efficacies. The lower the efficacy of the drug the lower the maximum response and slope of the log concentration–response curve. (B) The relationship between response and receptor occupancy for the series. Note that the full agonist, phenylephrine, produces a near-maximal response when only about half the receptors are occupied, whereas partial agonists produce submaximal responses even when occupying all of the receptors. The efficacy of tolazoline is so low that it is classified as an α-adrenoceptor antagonist (see Ch. 15). In these experiments, receptor occupancy was not measured directly, but was calculated from pharmacological estimates of the equilibrium constants of the drugs. (Data from Ruffolo, R.R. Jr, et al., 1979. J. Pharmacol. Exp. Ther. 209, 429–436.)

strips of rabbit aorta. The full agonist **phenylephrine** produced the maximal response of which the tissue was capable; the other compounds could only produce submaximal responses and are partial agonists. The difference between full and partial agonists lies in the relationship between receptor occupancy and response. In the experiment shown in Fig. 2.7 it was possible to estimate the affinity of the various drugs for the receptor, and hence (based on the theoretical model described later; p. 19) to calculate the fraction of receptors occupied (known as *occupancy*) as a function of drug concentration. Plots of response as a function of occupancy for the different compounds are shown in Fig. 2.7B, showing that for partial agonists the response at a given level of occupancy is less than for full agonists. The weakest partial agonist, **tolazoline**, produces a barely detectable response even at 100% occupancy, and is usually classified as a *competitive antagonist* (see p. 10 and Ch. 15).

These differences can be expressed quantitatively in terms of *efficacy* (e), a parameter originally defined by Stephenson (1956) that describes the 'strength' of the agonist–receptor complex in evoking a response of the tissue. In the simple scheme shown in Fig. 2.1, efficacy describes the tendency of the drug–receptor complex to adopt the active (AR*), rather than the resting (AR), state. A drug with zero efficacy ($e = 0$) has no tendency to cause receptor activation, and causes no tissue response. A full agonist is a drug whose efficacy[5] is sufficient that it produces a maximal response when less than 100% of receptors are occupied. A partial agonist has lower efficacy, such that 100% occupancy elicits only a submaximal response.

▼ Subsequently it was appreciated that efficacy is composed of drug-dependent and tissue-dependent components. The drug-dependent component is referred to as the *intrinsic efficacy*, which is the ability of the agonist drug molecule, once bound, to activate the receptor protein (see Kelly, 2013). The tissue-dependent components of efficacy include the number of receptors that it expresses and the efficiency of coupling of receptor activation to the measured tissue response. The number of receptors expressed is especially relevant to the study of receptors in recombinant expression systems when receptors are often very highly expressed and intermediate efficacy agonists then appear as full agonists. Across different cell types expressing the same receptor but at different densities a given drug of intermediate efficacy may appear as a full agonist in one tissue (high level of receptor expression), a partial agonist in another (lower level of receptor expression), and even as an antagonist in another (very low level of receptor expression). The term 'partial agonist' is therefore only applicable when describing the action of a drug on a specific tissue or cell type.

For G protein–coupled receptors the elucidation of their X-ray crystal structures (described in Ch. 3) and the application of molecular dynamic simulations of drug binding are beginning to tease out the molecular basis of receptor activation and why some ligands are agonists and some are antagonists. For students starting to study pharmacology the simple theoretical two-state model described below provides a useful starting point.

PARTIAL AGONISTS AS ANTAGONISTS

In discussing the efficacy of partial agonists above we considered the situation in which the tissue was exposed

[5]In Stephenson's formulation, efficacy is the reciprocal of the occupancy needed to produce a 50% maximal response, thus $e = 25$ implies that a 50% maximal response occurs at 4% occupancy. There is no theoretical upper limit to efficacy.

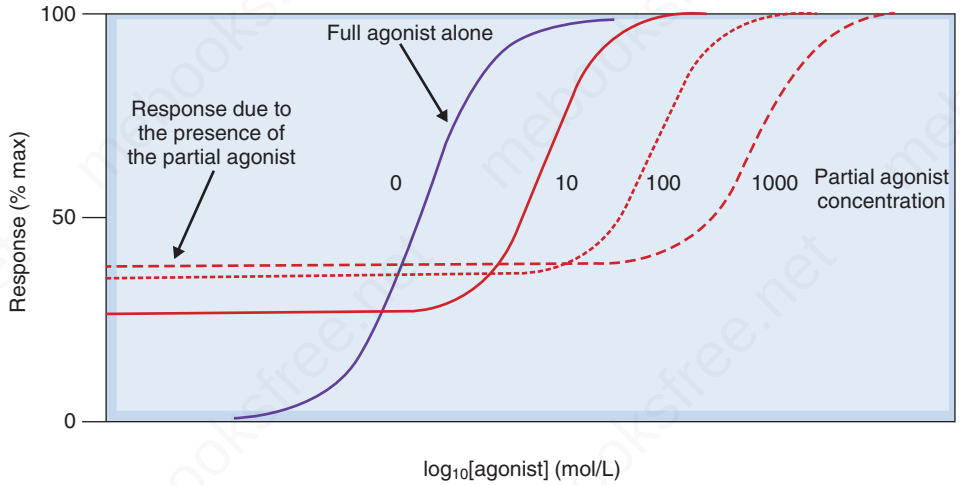

Fig. 2.8 Hypothetical concentration–response curves for a full agonist in the absence and presence of increasing concentrations of a partial agonist. The partial agonist will have agonist action and hence the initial response increases as the partial agonist concentration increases, reaching a maximum equal to the maximum response of the partial agonist. However, when the full agonist is added in the presence of the partial agonist its concentration–response curve is shifted to the right.

to only one drug, the partial agonist. What we should also consider is how the presence of a partial agonist would alter the response of a tissue to a higher efficacy agonist. This is depicted in Fig. 2.8 where it can be seen that the presence of the partial agonist induces some level of response dependent upon the concentration initially applied but in addition because the partial agonist is competing with the full agonist for the receptors it effectively acts as a competitive antagonist, shifting the concentration–response curve of the full agonist to the right. This is not just an obscure theoretical point but something which occurs in clinical practice. In the treatment of heroin users, buprenorphine, a weak partial agonist, not only acts as a weak opioid substitute but also acts as an antagonist and reduces the likelihood of overdose when users relapse and take heroin again (see Ch. 50).

CONSTITUTIVE RECEPTOR ACTIVATION AND INVERSE AGONISTS

▼ Although we are accustomed to thinking that receptors are activated only when an agonist molecule is bound, there are examples (see De Ligt et al., 2000) where an appreciable level of activation (*constitutive activation*) may exist even when no ligand is present. These include receptors for benzodiazepines (see Ch. 45), cannabinoids (Ch. 20), serotonin (Ch. 16) and several other mediators. Furthermore, receptor mutations occur – either spontaneously, in some disease states (see Bond & Ijzerman, 2006), or experimentally created (see Ch. 4) – that result in appreciable constitutive activation. If a ligand reduces the level of constitutive activation; such drugs are known as *inverse agonists* (Fig. 2.9; see De Ligt et al., 2000) to distinguish them from *neutral antagonists*, which do not by themselves affect the level of activation. Inverse agonists can be regarded as drugs with negative efficacy, to distinguish them from agonists (positive efficacy) and neutral antagonists (zero efficacy). Neutral antagonists, by binding to the agonist binding site, will antagonise both agonists and inverse agonists. Inverse agonism was first observed at the benzodiazepine receptor (Ch. 45) but such drugs are proconvulsive and thus not therapeutically useful! New examples of constitutively active receptors and inverse agonists are emerging with increasing frequency (mainly among G protein–coupled receptors). **Pimavanserin**, an inverse agonist at the 5-HT_{2A} receptor, has recently been developed for the treatment of psychosis associated with Parkinson's disease (see Chs 41 and 47). It turns out that most of the receptor antagonists in clinical use are actually inverse agonists when tested in systems showing constitutive receptor activation. However, most receptors – like cats – show a preference for the inactive state, and for these there is no practical difference between a competitive antagonist and an inverse agonist. The following section describes a simple model that explains full, partial and inverse agonism in terms of the relative affinity of different ligands for the resting and activated states of the receptor.

The two-state receptor model

▼ As illustrated in Fig. 2.1, agonists and antagonists both bind to receptors, but only agonists activate them. How can we express this difference, and account for constitutive activity, in theoretical terms? The two-state model (Fig. 2.10) provides a simple but useful approach. As shown in Fig. 2.1, we envisage that the occupied receptor can switch from its 'resting' (R) state to an activated (R*) state, R* being favoured by binding of an agonist but not an antagonist molecule. As described above, receptors may show constitutive activation (i.e. the R* conformation can exist without any ligand being bound), so the added drug encounters an equilibrium mixture of R and R* (see Fig. 2.10). If it has a higher affinity for R* than for R, the drug will cause a shift of the equilibrium towards R* (i.e. it will promote activation and be classed as an agonist). If its preference for R* is very large, nearly all the occupied receptors will adopt the R* conformation and the drug will be a full agonist; if it shows only a modest degree of selectivity for R* (say 5- to 10-fold), a smaller proportion of occupied receptors will adopt the R* conformation and it will be a partial agonist; if it shows no preference, the prevailing R : R* equilibrium will not be disturbed and the drug will be a neutral antagonist (zero efficacy), whereas if it shows selectivity for R it will shift the equilibrium towards R and be an inverse agonist (negative efficacy). We can therefore think of efficacy as a property determined by the relative affinity of a ligand for R and R*, a formulation known as the *two-state model*, which is useful in that it puts a physical interpretation on the otherwise mysterious meaning of efficacy, as well as accounting for the existence of inverse agonists.

BIASED AGONISM

A major problem with the two-state model is that, as we now know, receptors are not actually restricted to two distinct states but have much greater conformational

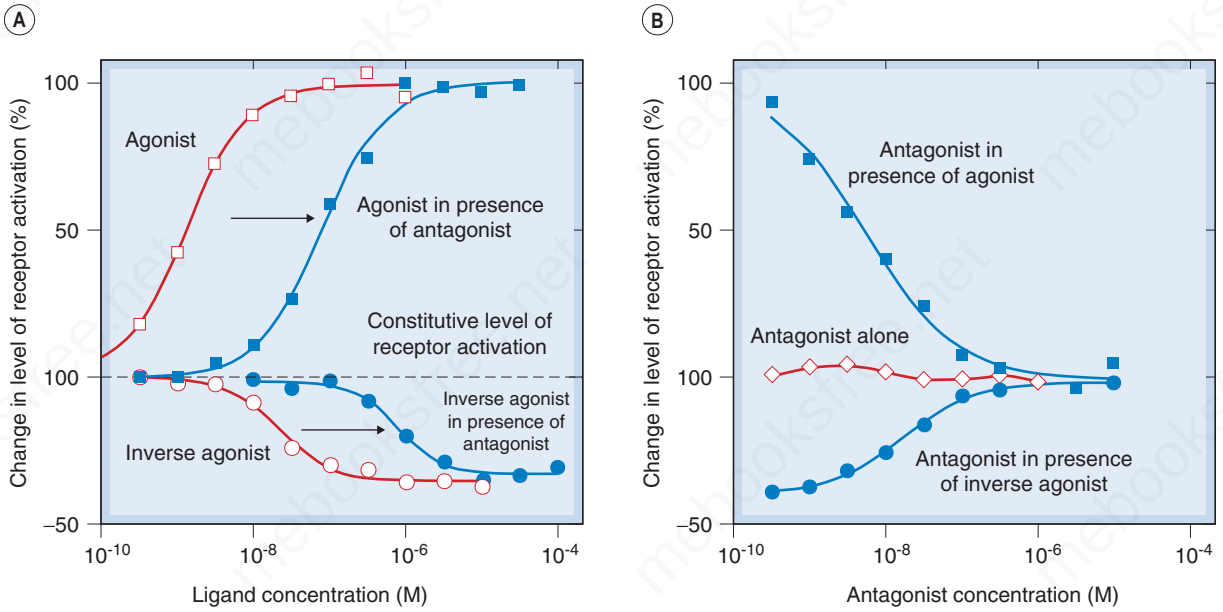

Fig. 2.9 Inverse agonism. The interaction of a competitive antagonist with normal and inverse agonists in a system that shows receptor activation in the absence of any added ligands (constitutive activation). (A) The degree of receptor activation (vertical scale) increases in the presence of an agonist *(open squares)* and decreases in the presence of an inverse agonist *(open circles)*. Addition of a competitive antagonist shifts both curves to the right *(closed symbols)*. (B) The antagonist on its own does not alter the level of constitutive activity *(open symbols)*, because it has equal affinity for the active and inactive states of the receptor. In the presence of an agonist *(closed squares)* or an inverse agonist *(closed circles)*, the antagonist restores the system towards the constitutive level of activity. These data (reproduced with permission from Newman-Tancredi, A., et al., 1997. Br. J. Pharmacol. 120, 737–739) were obtained with cloned human 5-hydroxytryptamine (5-HT) receptors expressed in a cell line. (Agonist, 5-carboxamidotryptamine; inverse agonist, spiperone; antagonist, WAY 100635; ligand concentration [M = mol/L]; see Ch. 16 for information on 5-HT receptor pharmacology.)

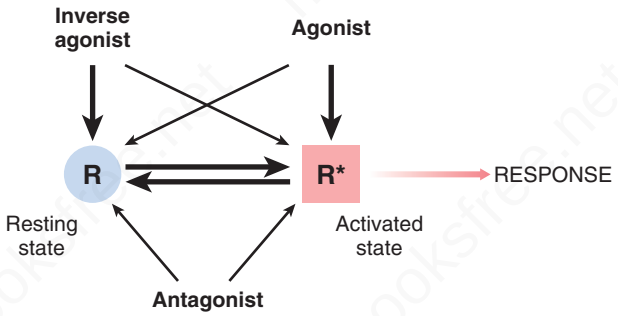

Fig. 2.10 The two-state model. The receptor is shown in two conformational states, *resting (R)* and *activated (R*)*, which exist in equilibrium. Normally, when no ligand is present, the equilibrium lies far to the left, and few receptors are found in the R* state. For constitutively active receptors, an appreciable proportion of receptors adopt the R* conformation in the absence of any ligand. Agonists have higher affinity for R* than for R, so shift the equilibrium towards R*. The greater the relative affinity for R* with respect to R, the greater the efficacy of the agonist. An inverse agonist has higher affinity for R than for R* and so shifts the equilibrium to the left. A *neutral* antagonist has equal affinity for R and R* so does not by itself affect the conformational equilibrium but reduces by competition the binding of other ligands.

flexibility, so that there is more than one inactive and active conformation. The different conformations that they can adopt may be preferentially stabilised by different ligands, and may produce different functional effects by activating different signal transduction pathways (see Ch. 3).

Receptors that couple to second messenger systems (see Ch. 3) can couple to more than one intracellular effector pathway, giving rise to two or more simultaneous responses. One might expect that all agonists that activate the same receptor type would evoke the same array of responses (Fig. 2.11A). However, it has become apparent that different agonists can exhibit bias for the generation of one response over another even though they are acting through the same receptor (Fig. 2.11B), probably because they stabilise different activated states of the receptor (see Kelly, 2013). Agonist bias has become an important concept in pharmacology.

Redefining and attempting to measure agonist efficacy for such a multistate model is problematic, however, and requires a more complicated state transition model than the two-state model described above. The errors, pitfalls and a possible way forward have been outlined by Kenakin & Christopoulos (2013).

ALLOSTERIC MODULATION

▼ In addition to the agonist binding site (now referred to as the *orthosteric* binding site), to which competitive antagonists also bind, receptor proteins possess many other (*allosteric*) binding sites (see Ch. 3) through which drugs can influence receptor function in various ways, by increasing or decreasing the affinity of agonists

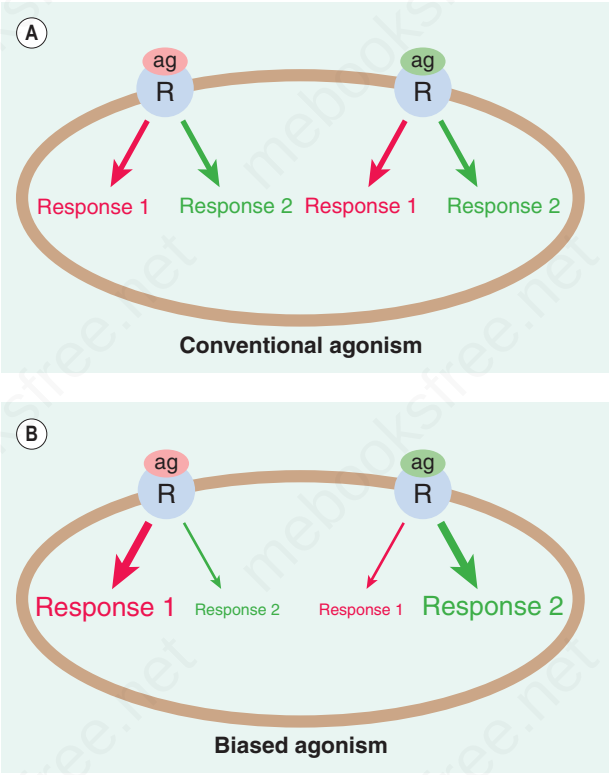

Fig. 2.11 Biased agonism. In (A), the receptor *(R)* is coupled to two intracellular responses – *response 1* and *response 2*. When different agonists indicated in *red* and *green* activate the receptor they evoke both responses in a similar manner. This is what we can consider as being conventional agonism. In (B), biased agonism is illustrated in which two agonists bind at the same site on the receptor yet the *red* agonist is better at evoking response 1 and the *green* agonist is better at evoking response 2.

for the agonist binding site, by modifying efficacy or by producing a response themselves (Fig. 2.12). Depending on the direction of the effect, the ligands may be allosteric antagonists or allosteric facilitators of the agonist effect, and the effect may be to alter the slope and maximum of the agonist log concentration–effect curve (see Fig. 2.12). This type of allosteric modulation of receptor function has attracted much attention recently and occurs at different types of receptors (see review by Changeux & Christopoulos, 2016). Well-known examples of allosteric facilitation include glycine at NMDA receptors (Ch. 39), benzodiazepines at $GABA_A$ receptors (Ch. 45) and **cinacalcet** at the Ca^{2+} receptor (Ch. 37). One reason why allosteric modulation may be important to the pharmacologist and future drug development is that across families of receptors such as the muscarinic receptors (see Ch. 14) the orthosteric binding sites are very similar and it has proven difficult to develop selective agonists and antagonists for individual subtypes. The hope is that there will be greater variation in the allosteric sites and that receptor-selective allosteric ligands can be developed. Furthermore, positive allosteric modulators will exert their effects only on receptors that are being activated by endogenous ligands and have no effect on those that are not activated. This might provide a degree of selectivity (e.g. in potentiating spinal inhibition mediated by endogenous opioids, see Ch. 43) and a reduction in side effect profile.

OTHER FORMS OF DRUG ANTAGONISM

Other mechanisms can also account for inhibitory interactions between drugs.

Agonists, antagonists and efficacy

- Drugs acting on receptors may be *agonists* or *antagonists*.
- Agonists initiate changes in cell function, producing effects of various types; antagonists bind to receptors without initiating such changes.
- Agonist potency depends on two parameters: *affinity* (i.e. tendency to bind to receptors) and *efficacy* (i.e. ability, once bound, to initiate changes that lead to effects).
- For antagonists, efficacy is zero.
- *Full agonists* (which can produce maximal effects) have high efficacy; *partial agonists* (which can produce only submaximal effects) have intermediate efficacy.
- According to the two-state model, efficacy reflects the relative affinity of the compound for the resting and activated states of the receptor. Agonists show selectivity for the activated state; antagonists show no selectivity. This model, although helpful, fails to account for the complexity of agonist action.
- *Inverse agonists* show selectivity for the resting state of the receptor, this being of significance only in situations where the receptors show *constitutive activity*.
- *Allosteric modulators* bind to sites on the receptor other than the agonist binding site and can modify agonist activity.

The most important ones are:

- chemical antagonism
- pharmacokinetic antagonism
- block of receptor–response linkage
- physiological antagonism

CHEMICAL ANTAGONISM

Chemical antagonism refers to the uncommon situation where the two substances combine in solution; as a result, the effect of the active drug is lost. Examples include the use of chelating agents (e.g. **dimercaprol**) that bind to heavy metals and thus reduce their toxicity, and the use of the neutralising antibody **infliximab**, which has an anti-inflammatory action due to its ability to sequester the inflammatory cytokine tumour necrosis factor (TNF; see Ch. 19).

PHARMACOKINETIC ANTAGONISM

Pharmacokinetic antagonism describes the situation in which the 'antagonist' effectively reduces the concentration of the active drug at its site of action. This can happen in various ways. The rate of metabolic degradation of the active drug may be increased (e.g. the reduction of the anticoagulant effect of **warfarin** when an agent that accelerates its hepatic metabolism, such as **phenytoin**, is given; see Chs 10 and 58). Alternatively, the rate of absorption of the active drug from the gastrointestinal tract may be reduced, or the rate of renal excretion may be increased. Interactions of this sort, discussed in more detail in Chapter 58, are common and can be important in clinical practice.

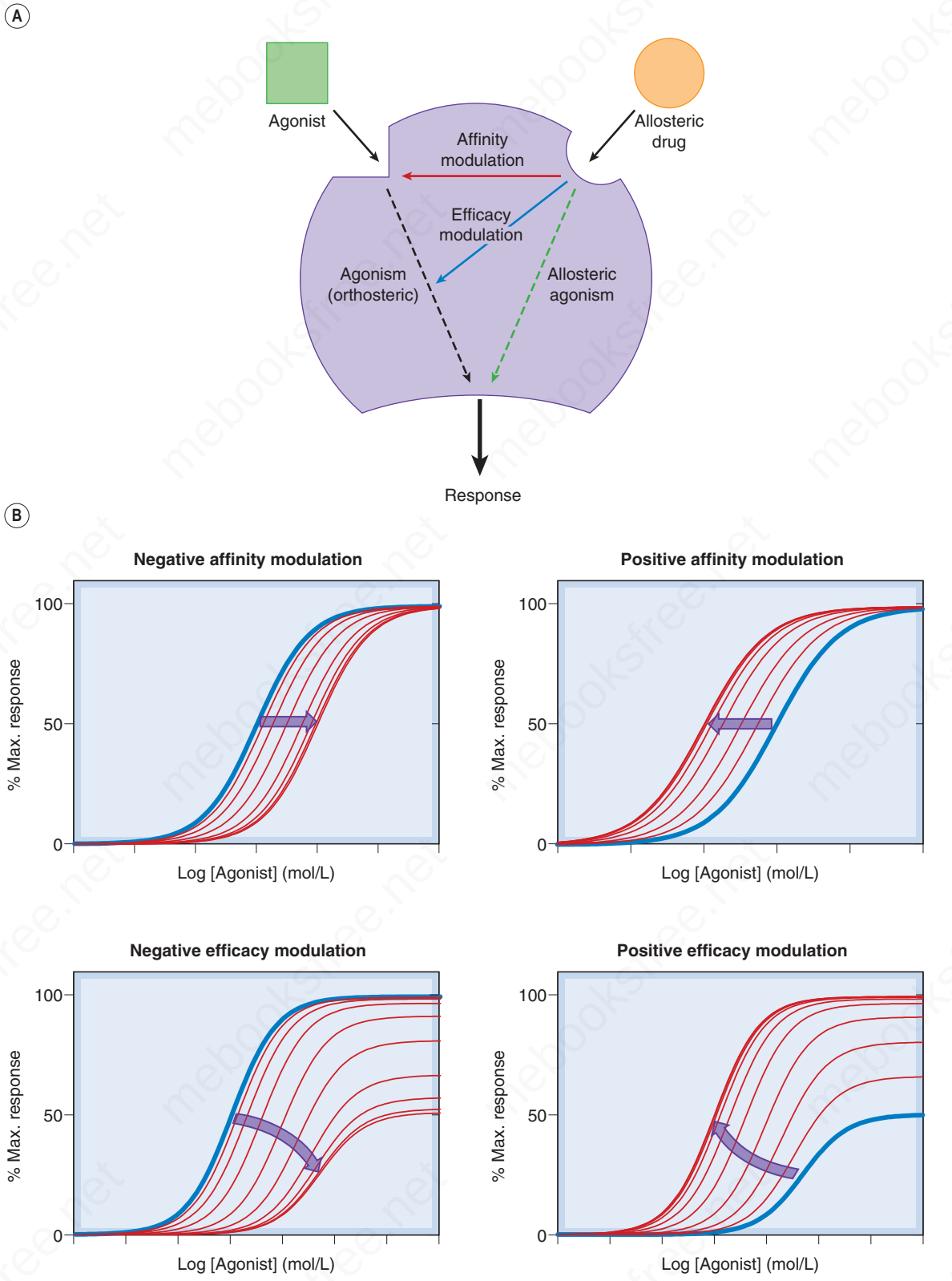

Fig. 2.12 Allosteric modulation. (A) Allosteric drugs bind at a separate site on the receptor to 'traditional' agonists (now often referred to as 'orthosteric' agonists). They can modify the activity of the receptor by (i) altering agonist affinity, (ii) altering agonist efficacy or (iii) directly evoking a response themselves. (B) Effects of affinity- and efficacy-modifying allosteric modulators on the concentration–effect curve of an agonist *(blue line)*. In the presence of the allosteric modulator the agonist concentration–effect curve *(now illustrated in red)* is shifted in a manner determined by the type of allosteric modulator until a maximum effect of the modulator is reached. (Panel [A] adapted with permission from Conn et al., 2009. Nat. Rev. Drug Discov. 8, 41–54; panel [B] courtesy of Christopoulos, A.)

BLOCK OF RECEPTOR–RESPONSE LINKAGE

Non-competitive antagonism describes the situation where the antagonist blocks at some point downstream from the agonist binding site on the receptor, and interrupts the chain of events that leads to the production of a response by the agonist. For example, **ketamine** enters the ion channel pore of the NMDA receptor (see Ch. 39) blocking it, thus preventing ion flux through the channels. Drugs such as **verapamil** and **nifedipine** prevent the influx of Ca^{2+} through the cell membrane (see Ch. 23) and thus non-selectively block the contraction of smooth muscle produced by drugs acting at any receptor that couples to these calcium channels. As a rule, the effect will be to reduce the slope and maximum of the agonist log concentration–response curve, although it is quite possible for some degree of rightward shift to occur as well.

PHYSIOLOGICAL ANTAGONISM

Physiological antagonism is a term used loosely to describe the interaction of two drugs whose opposing actions in the body tend to cancel each other. For example, **histamine** acts on receptors of the parietal cells of the gastric mucosa to stimulate acid secretion, while **omeprazole** blocks this effect by inhibiting the proton pump; the two drugs can be said to act as physiological antagonists.

Types of drug antagonism

Drug antagonism occurs by various mechanisms:

- chemical antagonism (interaction in solution)
- pharmacokinetic antagonism (one drug affecting the absorption, metabolism or excretion of the other)
- competitive antagonism (both drugs binding to the same receptors); the antagonism may be reversible or irreversible
- interruption of receptor–response linkage
- physiological antagonism (two agents producing opposing physiological effects)

DESENSITISATION AND TOLERANCE

Often, the effect of a drug gradually diminishes when it is given continuously or repeatedly. *Desensitisation* and *tachyphylaxis* are synonymous terms used to describe this phenomenon, which often develops in the course of a few minutes. The term *tolerance* is conventionally used to describe a more gradual decrease in responsiveness to a drug, taking hours, days or weeks to develop, but the distinction is not a sharp one. The term *refractoriness* is also sometimes used, mainly in relation to a loss of therapeutic efficacy. *Drug resistance* is a term used to describe the loss of effectiveness of antimicrobial or antitumour drugs (see Chs 51 and 57). Many different mechanisms can give rise to these phenomena. They include:

- change in receptors
- translocation of receptors
- exhaustion of mediators
- increased metabolic degradation of the drug
- physiological adaptation

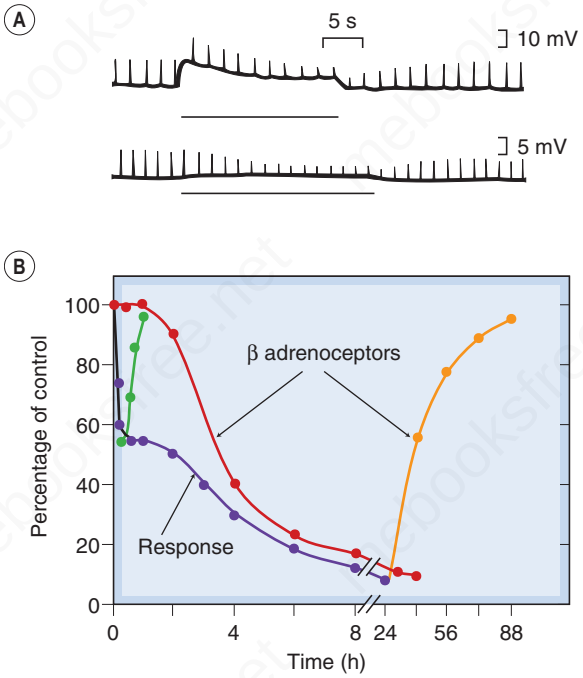

Fig. 2.13 Two kinds of receptor desensitisation. (A) Acetylcholine (ACh) at the frog motor endplate. Brief depolarisations *(upward deflections)* are produced by short pulses of ACh delivered from a micropipette. A long pulse *(horizontal line)* causes the response to decline with a time course of about 20 seconds, owing to desensitisation, and it recovers with a similar time course. (B) β adrenoceptors of rat glioma cells in tissue culture. Isoproterenol (1 μmol/L) was added at time zero, and the adenylyl cyclase response and β-adrenoceptor density measured at intervals. During the early uncoupling phase, the response *(blue line)* declines with no change in receptor density *(red line)*. Later, the response declines further concomitantly with disappearance of receptors from the membrane by internalisation. The *green* and *orange lines* show the recovery of the response and receptor density after the isoproterenol is washed out during the early or late phase. (Panel [A] from Katz B., Thesleff S., 1957. J. Physiol. 138, 63; panel [B] from Perkins, J.P., 1981. Trends Pharmacol. Sci. 2, 326.)

- active extrusion of drug from cells (mainly relevant in cancer chemotherapy; see Ch. 57)

CHANGE IN RECEPTORS

Among receptors directly coupled to ion channels (see Ch. 3), desensitisation is often rapid and pronounced. At the neuromuscular junction (Fig. 2.13A), the desensitised state is caused by a conformational change in the receptor, resulting in tight binding of the agonist molecule without the opening of the ionic channel. Phosphorylation of intracellular regions of the receptor protein is a second, slower mechanism by which ion channels become desensitised.

Most G protein–coupled receptors (see Ch. 3) also show desensitisation (Fig. 2.13B). Phosphorylation of the receptor interferes with its ability to activate second messenger cascades, although it can still bind the agonist molecule. The molecular mechanisms of this 'uncoupling' are considered further in Chapter 3. This type of desensitisation

usually takes seconds to minutes to develop, and recovers when the agonist is removed.

It will be realised that the two-state model in its simple form, discussed earlier, needs to be further elaborated to incorporate additional desensitised states of the receptor.

TRANSLOCATION OF RECEPTORS

Prolonged exposure to agonists often results in a gradual decrease in the number of receptors expressed on the cell surface, as a result of *internalisation* of the receptors. This is shown for β adrenoceptors in Fig. 2.13B and is a slower process than the uncoupling described above. Similar changes have been described for other types of receptor, including those for various peptides. The internalised receptors are taken into the cell by endocytosis of patches of the membrane, a process that normally depends on receptor phosphorylation and the subsequent binding of *arrestin* proteins to the phosphorylated receptor (see Ch. 3, Fig. 3.16). This type of adaptation is common for hormone receptors and has obvious relevance to the effects produced when drugs are given for extended periods. It is generally an unwanted complication when agonist drugs are used clinically.

EXHAUSTION OF MEDIATORS

In some cases, desensitisation is associated with depletion of an essential intermediate substance. Drugs such as **amphetamine**, which acts by releasing amines from nerve terminals (see Chs 15 and 49), show marked tachyphylaxis because the amine stores become depleted.

ALTERED DRUG METABOLISM

Tolerance to some drugs, for example **barbiturates** and **ethanol** (Ch. 49), occurs partly because repeated administration of the same dose produces a progressively lower plasma concentration, as a result of increased metabolic degradation. The degree of tolerance that results is generally modest, and in both of these examples other mechanisms contribute to the substantial tolerance that actually occurs. However, the pronounced tolerance to **nitrovasodilators** (see Chs 21 and 23) results mainly from decreased metabolism, which reduces the release of the active mediator, nitric oxide.

PHYSIOLOGICAL ADAPTATION

Diminution of a drug's effect may occur because it is nullified by a homeostatic response. For example, the blood pressure-lowering effect of **thiazide diuretics** is limited because of a gradual activation of the renin–angiotensin system (see Ch. 23). Such homeostatic mechanisms are very common, and if they occur slowly the result will be a gradually developing tolerance. It is a common experience that many side effects of drugs, such as nausea or sleepiness, tend to subside even though drug administration is continued. We may assume that some kind of physiological adaptation is occurring, presumably associated with altered gene expression resulting in changes in the levels of various regulatory molecules, but little is known about the mechanisms involved.

QUANTITATIVE ASPECTS OF DRUG–RECEPTOR INTERACTIONS

▼ Here we present some aspects of so-called receptor theory, which is based on applying the Law of Mass Action to the drug–receptor interaction and which has served well as a framework for interpreting a large body of quantitative experimental data (see Colquhoun, 2006).

THE BINDING REACTION

▼ The first step in drug action on specific receptors is the formation of a reversible drug–receptor complex, the reactions being governed by the Law of Mass Action. Suppose that a piece of tissue, such as heart muscle or smooth muscle, contains a total number of receptors, N_{tot}, for an agonist such as adrenaline. When the tissue is exposed to adrenaline at concentration x_A and allowed to come to equilibrium, a certain number, N_A, of the receptors will become occupied, and the number of vacant receptors will be reduced to $N_{tot} - N_A$. Normally, the number of adrenaline molecules applied to the tissue in solution greatly exceeds N_{tot}, so that the binding reaction does not appreciably reduce x_A. The magnitude of the response produced by the adrenaline will be related (even if we do not know exactly how) to the number of receptors occupied, so it is useful to consider what quantitative relationship is predicted between N_A and x_A. The reaction can be represented by:

$$\begin{array}{ccccc} A & + & R & \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} & AR \\ \text{drug} & + & \text{free receptor} & & \text{complex} \\ (x_A) & & (N_{tot} - N_A) & & (N_A) \end{array}$$

The Law of Mass Action (which states that the rate of a chemical reaction is proportional to the product of the concentrations of reactants) can be applied to this reaction.

$$\text{Rate of forward reaction} = k_{+1} x_A (N_{tot} - N_A) \quad (2.1)$$

$$\text{Rate of backward reaction} = k_{-1} N_A \quad (2.2)$$

At equilibrium, the two rates are equal:

$$k_{+1} x_A (N_{tot} - N_A) = k_{-1} N_A \quad (2.3)$$

The *affinity constant* of binding is given by k_{+1}/k_{-1} and from Eq. 2.3 equals $N_A/x_A(N_{tot} - N_A)$. Unfortunately, this has units of reciprocal concentration (L/mol) which for some of us is a little hard to get our heads around. Pharmacologists therefore tend to use the reciprocal of the affinity constant, the *equilibrium dissociation constant* (K), which has units of concentration (mol/L).

For drug A its equilibrium dissociation constant (K_A)[6] can be represented as

$$K_A = k_{-1}/k_{+1} = x_A (N_{tot} - N_A)/N_A \quad (2.4)$$

The proportion of receptors occupied, or occupancy (P_A), is N_A/N_{tot}, which is independent of N_{tot}.

$$P_A = \frac{x_A}{x_A + k_{-1}/k_{+1}} = \frac{x_A}{x_A + K_A} \quad (2.5)$$

Thus if the equilibrium dissociation constant of a drug is known we can calculate the proportion of receptors it will occupy at any concentration.

Eq. 2.5 can be written:

$$P_A = \frac{x_A/K_A}{x_A/K_A + 1} \quad (2.6)$$

This important result is known as the Hill–Langmuir equation.[7]

[6]Here we now use 'K_A' rather than just 'K' because we will in the next section be going on to consider the situation when two drugs, A and B, are present and there we will use 'K_A' and 'K_B' to denote the equilibrium dissociation constants of the two drugs.

[7]A.V. Hill first published it in 1909, when he was still a medical student. Langmuir, a physical chemist working on gas adsorption, derived it independently in 1916. Both subsequently won Nobel Prizes. Until recently, it was known to pharmacologists as the Langmuir equation, even though Hill deserves the credit.