An Introduction to Mechanisms in Pharmacology and Therapeutics

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Preface

This book is primarily intended for preclinical and clinical medical students and those preparing for an honours B.Sc degree in Pharmacology. It is also hoped that it will be of use to all those interested in understanding how drugs work. It is not meant to replace existing textbooks in Pharmacology which deal systematically with the general properties of drugs, but to provide some extra information and background reading on modes of action of drugs. In some ways the book attempts to illustrate how it may be possible for this aspect of Pharmacology to form a bridge between cell biology and the practice of clinical medicine.

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School, London, 1976.

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1 The Cell and Drugs

A drug is a substance which can alter the function of living cells. In the majority of cases the precise mode of action of drugs is not understood. However much fragmentary information exists and with an increasing number of drugs this is being integrated to reveal a wide range of possible mechanisms of drug action. Many drugs act at remarkably low concentrations, but even small modifications of their molecular structure may result in a diminution or abolition of their effects on cells. This suggests that the target regions for a particular drug in the cell may occupy a relatively small number of sites and that for chemicals to act on these areas a highly specific reaction between chemical groups in the drug and cellular target must occur.

Action of drugs on cell organelles

The surface membrane of the cell is not only a barrier which separates the relatively constant cell interior from the fluid in which it lies, but is also the first part of the cell to detect and respond to alterations in this external fluid environment. Many drugs, including acetylcholine, adrenaline and noradrenaline, appear to act primarily on the cell membrane. Also it appears that their antagonists–such as atropine, d-tubocurarine, propanolol and phentolamine act by competing for the same specific receptor sites on the cell membrane. Pharmacological agents may also act on other cellular organelles: the anti-tumour drug mustine binds to both helical strands of nuclear DNA and prevents replication of DNA which is a necessary step prior to mitosis. The anti-microbial drug streptomycin acts on bacterial ribosomes and inhibits protein synthesis by interfering with the translatory step by which the sequence of bases in mRNA is converted into the correct sequence of amino acids in the growing peptide chain. Mitochondrial oxidases are inhibited by the monoamine oxidase inhibitor drugs which are used to treat some forms of psychiatric depression. These agents also inhibit microsomal drug metabolising enzyme systems. Dinitrophenol interferes with other aspects of mitochondrial function in that it decreases the production of ATP by this organelle without a corresponding decrease in oxygen consumption. This is known as uncoupling of oxidative phosphorylation. The lysosomal membrane is stabilised by adrenal glucocorticoids–such as cortisol–and thus is made less likely to release its contents to

the extracellular fluid. Conversely, reserpine makes the storage vesicles in certain nerve terminals more leaky and in this way may deplete neuronal processes of neurotransmitter substances.

Molecular specificity of drug action

Very few drugs have a single action, nevertheless for a drug to produce its clinically important effect a highly specific interaction between the drug and a cellular component may be necessary. The antihistamine drug, diphenhydramine, for example, binds to several membrane proteins but its principal use is as an antihistamine and exhibits this property by binding to one type of histamine receptor in the plasma membrane of several cell types. The term receptor means the first component of a cell with which a drug comes into contact in order to initiate the sequence of events which eventually lead to the drug response. Diphenhydramine competes with histamine for its receptor and thus prevents some of the actions of histamine. In some instances the physical or chemical nature of the drug receptor has been characterised. Catecholamines such as adrenaline may stimulate the heart because of their potentiating action on myocardial adenyl cyclase. This results in the production of increased amounts of cAMP. cAMP activates phosphorylase which in turn breaks down glycogen to glucose-1-phosphate and thus provides more energy for cardiac contraction. Caffeine has a similar action on the heart, but produces this effect by inhibiting phosphodiesterase. This is the enzyme which normally attacks cAMP. The result is that cAMP levels will also be raised in the heart but the process is initiated by a different drug-protein interaction from that which occurs with the catecholamines.

Drugs may react with molecular species other than proteins. The anti tumour drugs actinomycin and mustine bind to guanine residues in DNA. The mode of action of such anaesthetic agents as ether or the alcohols is not understood, but there is evidence that they produce a swelling and disorganisation of the arrangement of molecules in the lipid moiety of cell membranes which are associated with changes in permeability properties. If such a change occurred in the nerve cells in the brain which are concerned with arousal, this could provide an explanation of the sleep inducing properties of these drugs.

The receptors for some drugs are proteins which are neither enzymic nor have been chemically defined. The muscarinic acetycholine receptor appears to be such a membrane protein which can be characterised and isolated only by the use of cholinergic drugs and their competitive antagonists. Almost nothing is known about the molecular nature of the sodium channel. This is the route through the cell membrane taken by sodium ions moving passively along an osmotic or electrical gradient. In some types of smooth muscle, glands, neurones and in the motor end

plate, acetylcholine apparently opens the sodium channel. This suggests an association between the acetylcholine receptor and the sodium channel. The sodium channel is selectively blocked by tetrodotoxin which is a substance found in the gonads and liver of the puffer fish and prevents depolarisation in the nerves of individuals who eat this fish. This produces numbness and paralysis which is presumably due to inhibition of transmembrane sodium fluxes which are an essential component of the electrical events occuring in the nerves.

Cellular specificity of drug action

The ability of drugs to influence one cell type differently from another is not only one of the characteristic features of drug action but is of vital importance in several therapeutic situations:

(a) In chemotherapy the aim is to destroy certain cells in a patient which are causing disease–such as cancer cells or micro-organisms–and yet leave the host unaffected. This can be attained by several methods. The host and parasite may show minor differences in corresponding enzymes which produce different susceptibilities to drugs. An example of this is the parasitic worm schistosoma, which is destroyed by stibophen. The parasite is harmed because the drug blocks glycolysis due to inhibition of the enzyme phosphofructokinase. Although the same metabolic step in man (fructose-6-phosphate +ATP → fructose-1-6-diphosphate) is also catalysed by phosphofructokinase, the patient does not show any impairment of tissue glycolysis. This selectivity arises because of small chemical differences between the enzymes in the two species which renders the host enzyme relatively unaffected by stibophen.

Similarly, the antibacterial drug trimethoprim acts by inhibiting dihydrofolate reductase. This enzyme is needed by both animal and bacterial cells for the synthesis of nucleic acids. However, to produce the same degree of inhibition of the mammaliam enzyme compared with the bacterial form, 50,000 times the concentration of trimethoprim is required. Thus using amounts of the drug in the body sufficient to harm bacteria, the enzyme systems of the host will be virtually unaltered.

An even more striking type of interspecies difference providing an opportunity for selective drug action is a total lack of the appropriate target in the cells of the host. Thus the sulphonamides are antibacterial because they inhibit the enzyme which converts p-amino benzoic acid to folic acid. The latter is necessary for the functioning of all cells. Animal cells lack the synthesising enzyme but have the ability to take up folic acid from the outside. Many bacteria cannot utilise exogenous folic acid but have to synthesise it intracellularly. It is these micro-organisms which sulphonamides will attack. These drugs will not harm animal cells as these do not possess the p-aminobenzoic acid metabolising enzyme.

A more tangible example of certain cells possessing specific targets for drug action is the bacterial cell wall. Animal cells possess no similar structure. Penicillin is one of several antibacterial agents which interfere with the synthesis of the bacterial cell wall. When susceptible bacteria are exposed to these drugs, an adequate cell wall is not made and the cell itself dies because of its lack of protection against osmotic and mechanical stresses.

Variations in permeability to the drug may also selectively protect the host against antibacterial agents. For instance chloramphenicol injures ribosomes both in bacteria and in animal mitochondria. The mitochondrial ribosomes are protected from injury as chloramphenicol cannot penetrate the intact mitochondrial membrane. There is no such permeability barrier protecting the bacterial ribosome and so the drug readily penetrates into the interior of the bacterial cell and blocks protein synthesis.

(b) Within the same animal different cells may appear to lack receptors for a particular drug. Thus although a gland may secrete or smooth muscle may contract when exposed to acetylcholine, fibroblasts do not obviously respond to this substance.

Different cells may possess different receptors for the same drug. Thus arteriolar muscle from voluntary muscles relax when exposed to adrenaline, whilst arteriolar cells from the skin contract when exposed to the same substance. In a similar way histamine increases capillary permeability and dilates arterioles (and these effects are antagonised by one sort of antagonist drug–H1 receptor blockers), and also increases gastric secretion (which is not antagonised by H1 blockers but by another class of compounds, the H2 receptor blockers).

Identical biochemical actions on different cells may obviously result in different types of response. The cardiac glycosides inhibit Na^+-, K^+-ATPase in many types of cell. In the red blood cell the drug produces a drop in the rate of active sodium extrusion with a subsequent rise in intracellular sodium and a fall in intracellular potassium content. When the heart is exposed to digoxin influx of sodium also occurs, but this releases sequestered calcium increasing the force of contraction of the heart during systole. Thus the fundamental potential of a cell for physiological response may provide a restriction for the type of pharmacological response it can give.

SUMMARY

A drug is a substance which can alter the function of living cells. The fact that drugs often act at minute concentrations and elicit highly specific responses in only certain cell types suggests that specific drug receptors may exist in cells. The cell specificity for different drugs means that in an animal one organ can be selectively influenced or that cells from one species can be acted on without any effect on another species. For drug receptor

interactions to occur precise and critical properties appear to be required both in drug and receptor. The molecular basis for cell or organelle specificity may be dependent on numerous molecular mechanisms including relatively small variations in corresponding molecules in the target of drug action; on the presence or absence of a structure or metabolic process which can be attacked; or on the relative accessibility of the similar targets in different tissues.

REFERENCES

Gale, E.F., Cundliffe, E., Reynolds, P.E., Richmond, M.M. and Waring, M.J. (1972) The molecular basis of antibiotic action. John Wiley, London.

Rang, H. P. (1971) Drug receptors and their action. *Nature*, 231: 91-96.

2 Drug-Receptor Interactions

The effects of drugs are, in the last analysis, the result of the interactions of drug molecules with the molecules composing the tissue. The idea that tissues might contain receptors for drugs was first put forward in 1878 by J. N. Langley in the first volume of the Journal of Physiology. He wrote concerning the opposite actions of atropine and pilocarpine on salivary flow in the cat: "We may, I think, without much rashness, assume that there is some substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors".

He later investigated the South American arrow poison curare following the work of Claude Bernard and showed that even in denervated muscle nicotine could produce a contraction and curare could block this chemically-induced contraction. He analysed this interaction and clearly had in mind the possibility of competition by these drugs for some specialised part of the muscle which he termed the receptor substance. At about the same time Paul Ehrlich, working in Germany on the then new science of chemotherapy, had discovered the high degree of specificity which was exhibited by chemotherapeutic substances for parasitic protozoa. Minor changes in the chemical structure produced major differences in the toxicity of these substances for both parasite and host. He had previously (in 1900) put forward a selective theory of antibody production postulating receptor bodies on the outside of the cells of the organism having 'haptophore groups' which were adapted to combine specifically with foreign proteins and toxins. Toxins were then thrown off of the cell having combined with these receptor bodies and disposed of by the body. It was therefore natural that he should have extended this concept to account for the effects of drugs on cells. He suggested that all cells had "receptors" which he saw as chemical groups on their surface. He defined a receptor as "that combining group of the protoplasmic molecule to which a foreign group, when introduced, attaches itself". Different cells possessed different receptors and therefore the aim of chemotherapy should be to design drugs which would combine specifically with the receptors of parasites but which have no affinity for the receptors of its host. He suggested that in the case of trypanosomes the receptor contained a mercapto group (–SH) and that the combination of this group with arsenic led to the death of the organism.

Ehrlich maintained that drugs could not act unless they were fixed in the tissues by combination with receptors. Whilst it is now believed that not all drugs act by combining with specific tissue receptors this doctrine was of sufficient validity to have given a firm basis for thinking about the actions of drugs in molecular terms for the next half century.

Nine years before Langley's work, Crum Brown and Frazer showed that by quaternising a number of alkaloids like morphine and strychnine with methyl iodide the characteristic properties of the alkaloids could be removed and replaced by a single curare-like action. They speculated that a relationship existed between the pharmacological activity of a substance and its chemical structure. From these beginnings have come the many studies of structure-activity relationships whereby subtle variations in the chemical structure of compounds are compared with the corresponding effect of such changes on pharmacological properties. This has allowed the recognition of "families" of compounds which owe their activities to certain structural groups. Examples of these families such as the phenothiazines or the barbiturates will be found throughout the text. Most drugs are, in chemical terms, relatively inert substances incapable of forming strong bonds with tissues. Yet, when added to a tissue preparation or injected into an animal these same unreactive molecules may produce startling effects. Many of these effects are reversible either in time, by the use of antagonists or merely by washing the drug away. Clearly the interaction between the drug and the tissue receptor does not involve irreversible fixation of one to the other. Many drugs such as adrenaline and atropine exist in optically active stereoisomers and although these isomers have identical chemical and physical properties they can differ markedly in their pharmacological activity. The shape of a molecule in 3-dimensional space is thus crucial to the union with the receptor and this suggests that the way in which a drug "recognises" its receptor is because they share a complementary structure. Some drugs may be surprisingly active and specific in their actions. Clark estimated that 0.02 μg of acetylcholine reduced the rate of contraction of an isolated frog heart by half. He showed that this was equivalent to covering about 0.016% of the surface of each ventricular cell with acetylcholine. More recently using a radioactively labelled irreversible antagonist which binds strongly to the acetylcholine (muscarinic) receptors of the smooth muscle cells of seminal vesicle it has been estimated that there are only 55,000 receptors per cell whereas for the rabbit aortic strip there are about 200,000 receptors per cell. Such activity could only be achieved if the drug were acting very precisely at certain sensitive areas on the surface of these cells i.e. at specific receptor sites. A few drugs are even more powerful: it has been calculated that only 1000 molecules of Botulinus type A toxin are required to kill a mouse.

A receptor may be defined as a tissue component fulfilling the following criteria:–

(a) it is a macromolecule bearing recognition sites for specific substances which bind to receptors and are sometimes referred to as ligands.

(b) the specificity of the receptor for ligands and the type of receptor response are genetically determined

(c) it is the ligand binding to the receptor which causes a specific change in the macromolecule and initiates a train of events which eventually produces a tissue response.

FORCES BINDING THE DRUG TO THE RECEPTOR

To understand the way in which drug and receptor structure complement each other and how such a specific interaction might lead to a biological event, the nature of the forces of interaction between these molecules must be understood.

(1) Electrostatic forces (sometimes called ionic bonds) exist between two charged particles and in a vacuum the energy of interaction ΔF is given by

$$\Delta F = \frac{Z_1 Z_2 e^2}{d}$$

where Z_1, Z_2 are the charge numbers (valencies) of the two particles

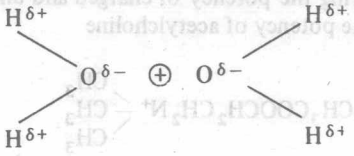
e is the charge

d is the distance between the charges

In biological systems the interactions occur in aqueous media of finite ionic strength and this relationship is modified by the dielectric constant D thus:

$$\Delta F = \frac{Z_1 Z_2 e^2}{dD}$$

However, the dielectric constant referred to here is not that of the bulk solution but a microscopic dielectric constant describing the properties of the medium in the immediate neighbourhood of the receptor. One reason for this alteration in properties is the dipole nature of water (i.e. there are positive and negative ends of the molecule) so that in the presence of a charge water molecules orientate themselves in preferred positions rather than randomly in the medium:



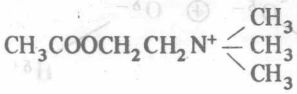
Because of this orientation the water molecules are unable to carry an electric charge so easily and the local dielectric constant in the neighbourhood of a charged area on a molecule will be depressed. It is found to fit the relationship $D = 6d - 7$ so that we can now write:

$$\Delta F = \frac{Z_1 Z_2 e^2}{d\,(6d - 7)}$$

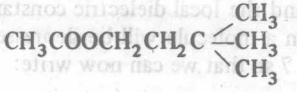
It will be noted that this relationship is now obeying an inverse square rule. The dielectric modification plays an important part in determining the strength of ionic interactions. Thus a pair of oppositely charged ions in vacuo will yield an interaction energy in excess of 100 Kcal/mole but in a biological system such interaction varies from 2–20 Kcal/mole depending on local factors. Making reasonable assumptions it would seem that at distances of less than 8Å the energy of interaction between a single pair of charges would be greater than the thermal free energy tending to disrupt the pair and certainly below 5Å ionic bonds could play a very significant role in drug-receptor binding.

In the physiological pH range proteins contain several groups which may be ionised e.g. terminal carboxyl groups, the carboxyl groups of aspartic and glutamic acids, arginine, lysine. Cationic amino sugars occur in mucopolysaccarides and other membrane components. The availability of such groups for bonding with drug molecules depends upon the tertiary structure of the receptor for some of these ionised groups will already be taking part in ionic interactions within the protein molecule or with other membrane components. Similarly drugs may contain cationic or anionic groupings capable of forming ionic bonds with oppositely charged groups in the receptor. Sometimes there are groups which possess a partially ionic character, e.g. the carboxyl group:

Some idea of the importance of charge in drug receptor interactions can be gained by comparing the potency of charged and uncharged analogues, e.g. comparison of the potency of acetylcholine



and its uncharged analogue



shows that the affinity of the former for its receptor is eight times that of its uncharged congener. Extensions of this method of comparing charged-uncharged pairs of molecules have been used to estimate distances between drug and receptor. There is one difficulty with this approach however, and that is the formation of a shell of water molecules around the charged areas of drug and receptor, because water molecules, being dipoles, line up around charges. Therefore charges on the drug and receptor can only come together by displacement of this bound water, which does not of course exist around the uncharged analogue. As yet a satisfactory theoretical scheme has not been worked out to compensate for these differences. Furthermore, in any interaction between drug and receptor it is uncertain how these two effects of ionic interaction and water displacement balance out.

(2) London dispersion forces (sometimes called van der Waals interactions or dipole-induced dipole bonds). These are very weak interactions between similar atoms but because of the abundance of carbon atoms in both drugs and receptors they may collectively exert significant influence on the binding of a drug to the receptor. On average the electronic charge distribution is spherically symmetrical around the nucleus of an atom. This is however only statistically true and at any instant of time the localisation of the various electrons will be into different orbitals dependent upon their energy. This asymmetrical distribution of charge will induce complementary changes of electronic charge distribution in neighbouring atoms and the result is a net attraction. However, as the two atoms approach, the cloud of electrons around each causes mutual repulsion and eventually overrides the induced dipolar attraction. The distance of separation at which the attractive force is maximal is the van der Waals contact distance and is a constant for the atoms involved since each atom has a specific van der Waals radius and the contact distance is merely the sum of the van der Waals radii for the atoms involved (See Fig. 1). The attractive and repulsive forces involved may be described by the equation:

$$\text{Net energy} = -\frac{A}{r^6} + \frac{B}{r^{12}}$$

where A and B are constants and r is the distance between the centres of the two atoms.

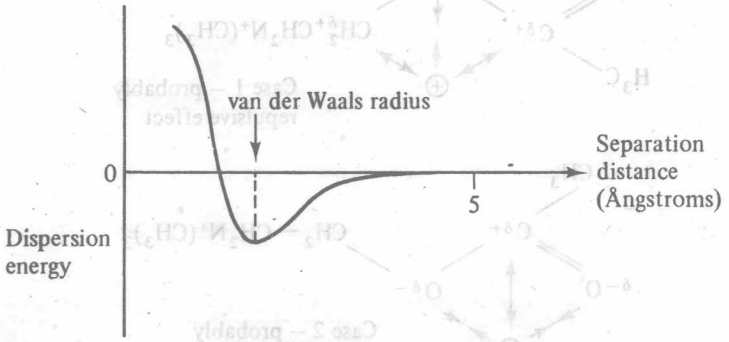


Fig. 1: Effect of interatomic distance on London dispersion forces. The van der Waals radius is the position of energy minimum.

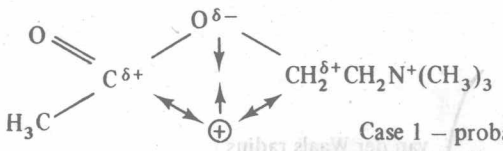
Clearly from this equation the effect of distance is critical and as a drug molecule approaches a receptor there will be a very sharp rise in attractive force as the interatomic distances decrease and the van der Waals contact distance is approached. If many atoms of drug and receptor can approach contact distance the energy of interaction will be large. This is because of the summation of many small forces of interaction. In order to achieve this situation there should be a complementary geometry of drug and receptor, because even minor degrees of misfit could substantially reduce the energy of interaction due to the great dependence of London dispersion forces on interatomic distance. Therefore the origins of the highly specific nature of drug receptor binding may be in this type of interaction.

(3) Dipolar interactions. These are interactions between polar molecules and the effect of distance on the energy involved is as shown:

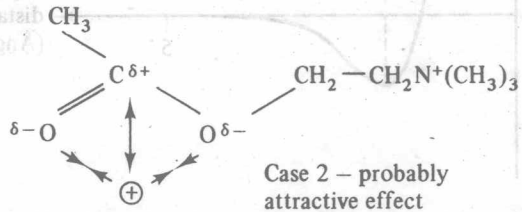
Ion – dipole	$\alpha\ 1/d^2$
Dipole – dipole	$\alpha\ 1/d^3$
Ion-induced dipole	$\alpha\ 1/d^4$

They are therefore intermediate between the inter-ionic forces on the one hand and the London dispersion forces on the other. Probably only the ion-dipole interaction plays any large part in drug receptor binding:

the other forces are too weak to influence events. Consider the dipole structure occurring with acetylcholine interacting with a charge on the receptor:



Case 1 – probably repulsive effect



Case 2 – probably attractive effect

Case 2 indicates a way by which this mechanism could act to bind agonist to receptor. Structure activity studies indicate that acetylcholine analogues lacking these two oxygen atoms have reduced agonist activity on a number of tissues which suggests that both these oxygens are important for the action of acetylcholine on its receptor. Calculations based on the above mechanism indicate that with a close fit of drug to receptor such dipolar interactions may be possible but of course taken by themselves they do not constitute complete proof that these forces are of importance in the drug receptor binding.

(4) Hydrogen bonding (sometimes called hydrophilic bonding). A proton can partially share an electron pair from a strong electron donor atom such as oxygen or nitrogen and in so doing create a weak bond (energy around 2-5 Kcals per mole). In a way this is a special case of a strong interaction between two dipoles. The additive effects of several of these bonds can stabilise intramolecular interactions. Important examples of this are the α helix of proteins and the helical structure of the nucleic acids. In the latter case hydrogen bonds are responsible not only for the specific complementary binding of adenine to thymidine, and guanine to cytosine, but also stabilise the whole molecule longitudinally.

Hydrogen bonds play an important role in drug solubility and in any equilibrium of the sort:

$$R_3N + H^+ \rightleftharpoons R_3NH^+$$