

# DRUG-RECEPTOR INTERACTION

H. R. ING

*Department of Pharmacology, University of Oxford, U.K.*

The only way of thinking about drug actions in chemical terms is to consider how the drug molecule alters the chemical properties or interferes with the biochemical reactions of living tissues. We can think about the drug only in physical or chemical terms; consequently, pharmacological events must also be interpreted in physical or chemical terms, and this ultimately means considering what happens *at the molecular level* when the drug impinges upon the highly complex chemical organization of living tissues.

I shall regard the drug as a chemical substance *foreign* to the animal body; of course, many substances normal to the animal body are commonly regarded as drugs, *e.g.*, noradrenaline, acetylcholine, insulin, and many other hormones and vitamins, but I think that investigation of their effects is really part of physiology, although it is relevant to pharmacology.

Now if we suppose, as we have good reason to, that the resting state of any organ or tissue is the result of a dynamic equilibrium between numerous biochemical reactions, continuously going on, we can regard the drug as a substance which interferes with, or disturbs the balance of, the normal state; and *a priori* we can distinguish two main ways in which drugs may do this.

(i) Drugs may alter the normal physicochemical state of living cells by a passive process, depending upon their possessing suitable physicochemical properties, such as lipoid solubility, surface active properties, *etc.*, and not upon a high degree of structural specificity. Effects of this kind are often referred to as "physical toxicity", and are characteristic of general anaesthetics, many narcotics and some disinfectants. It is usual for homologous series of such drugs to display a geometrical increase in activity (up to a limiting value) as the number of C-atoms increases arithmetically; in other words, activity behaves in the same way as any physical property which depends upon the distribution of the drug molecule between two phases<sup>1</sup>.

(ii) Drugs may owe their effects to the formation of some kind of chemical combination with particular tissue constituents. Such combinations may be true covalent ones, which may be more-or-less stable, or they may be complexes due to relatively weak forces, such as polar or ionic forces, hydrogen-bonding or dispersion (van der Waals or London) forces. Drugs which owe their pharmacological effects to chemical combination (of whatever kind) with tissue constituents are likely to have localized and specific effects, simply because they are able to combine with only a limited range of tissue constituents.

The tissue constituent with which a drug is supposed to combine *in order to produce a pharmacological effect* is called a "receptor". The receptor must be distinguished from other tissue constituents with which drugs may combine without producing any well-defined pharmacological effects, and which are commonly called "sites of loss".

We owe the idea of receptors to Langley<sup>2</sup> but Ehrlich was the first to give chemical precision to the idea; he thought in more purely chemical terms than Langley, and he drew an important distinction between haptophoric groups in a drug molecule that were responsible for the concentration of the drug at particular histological sites, and toxiphoric groups that were responsible for its therapeutic effect. Ehrlich founded the science of chemotherapy, but since pharmacologists have left this subject mainly to pathologists and chemists, they have frequently forgotten the distinction between haptophoric and toxiphoric groups.

The evidence for the existence of receptors is usually indirect. Unless the receptor can be identified as an enzyme, the evidence rests mainly upon the specificities—pharmacological, stereochemical and structural—of drugs.

If the receptor is an enzyme, it is usually possible to study the interaction of the drug with, at least, a crude preparation of the enzyme *in vitro*. But many receptors may be non-enzymic macromolecular constituents of living cells, or even small structural units in such macromolecules; consequently, *in vitro* studies may be misleading because the effect of the drug-receptor combination may depend upon a highly complex organization of macromolecules at the site upon which the drug acts. Numerous attempts have been made to localize receptors in cells by histological methods; *e.g.* by using a drug which was itself a dyestuff, or by converting a drug into a coloured substance *in situ*, or by autoradiographs of tissue slices taken from an organ previously treated with a radioactively labelled drug. Thus, if we confine ourselves to striated muscle, Fühner<sup>3</sup> used curarizing dyestuffs; Fulton<sup>4</sup> used procaine on frog muscle, diazotized the drug *in situ* and coupled the product with  $\beta$ -naphthol; Waser<sup>5</sup> made autoradiographs of sections of rat diaphragm, previously treated with labelled tubocurarine.

The difficulty about this kind of work is to prove a necessary connexion between the observed localization of the drug and its pharmacological effect, since the drug may be preferentially concentrated at "sites of loss"; thus, Fulton found that procaine was mainly concentrated in the nuclei of muscle cells, whereas its effect upon frog muscle was almost certainly exerted on the cell membrane. Even Waser's beautiful experiments only prove that tubocurarine is not significantly concentrated at sites other than the motor end-plate of rat diaphragm at which we know on other grounds that it must act. On the other hand, Chagas<sup>6</sup> found in cats and dogs that radioactively labelled curare alkaloids were concentrated at many non-specific sites (particularly cartilage), probably owing to salt formation with acidic mucopolysaccharides.

Isolation of receptor substances, unless they be enzymes, presents an even more difficult problem, because evidence that a drug forms a complex with a particular tissue constituent, such as a ganglioside, is no evidence that the characteristic action of the drug is due to complex formation with that particular tissue constituent, although it does indicate the kind of macromolecule with which it will combine. I do not wish to discourage attempts to isolate receptor molecules; I only wish to draw attention to the pitfalls into which investigators may fall.

A third way of approaching the problem of drug-receptor interaction is to consider the kinetics of drug actions. Clark<sup>7</sup> was the pioneer in this field.

## DRUG-RECEPTOR INTERACTION

He tried to put the drug-receptor concept upon a quantitative basis and he thought that he had done so by applying Langmuir's empirical absorption equation to dose-response curves. Clark's views necessarily involved two ideas: (i) that the pharmacological response depended upon an *equilibrium* between association of a drug with a receptor and dissociation of the drug-receptor complex; and (ii) that every drug-receptor combination led to an all-or-none response<sup>8</sup>. To Clark, drug action was a colligative property, depending on the *number* of individual drug-receptor combinations at any given moment.

Unfortunately, Clark's views led to a too rigid slope for dose-response curves. Both Stephenson<sup>9</sup> and Ariëns<sup>10</sup> have tried to get over this difficulty. Both authors have rejected the all-or-none response idea of the drug-receptor combination while retaining Clark's idea of an equilibrium between drug and receptor. Stephenson introduced the idea of the "efficacy" of a drug-receptor combination and Ariëns, adapting the Michaelis theory of enzyme action to drugs, distinguished between the affinity of a drug for a given receptor and the "intrinsic activity" of the drug-receptor complex. However, no chemical meaning can be given to the concepts of "efficacy" or "intrinsic activity".

Paton<sup>11</sup>, stimulated by his interest in the diphasic effects of nicotine on ganglia, has developed an entirely new approach to the kinetics of drug action. He has rejected Clark's idea of receptor occupation as the determining factor and has suggested that an excitatory pharmacological effect depends, not upon the *number* of individual drug-receptor combinations, but upon the *rate*,  $k_1$ , of drug-receptor occupation. Indeed, he assumes that receptor occupation itself immobilizes receptors, so that the observed pharmacological effect depends upon the ratio  $k_2/k_1$  where  $k_2$  is the rate of dissociation of drug-receptor complexes. When  $k_2$  is high the drug will be a powerful stimulant because receptors are rapidly set free from occupation; when  $k_2$  is low the drug will be mainly antagonistic, whereas intermediate values of  $k_2$  will characterize partial agonists.

At first sight it is difficult to see why a pharmacological effect should depend upon a ratio of rates of two opposing chemical reactions, but not upon an equilibrium; however, if the pharmacological effect is determined by, and is a measure of, the formation of an activated transition state of the drug-receptor complex, the difficulty may be overcome; "activated" drug-receptor combinations will be very short lived because of their high energy: either they will dissociate rapidly ( $k_2$  high) or they will form relatively stable drug-receptor complexes of lower energy ( $k_2$  low) which will block the receptors.

I do not wish to imply that my interpretation of Paton's rate theory would meet with his approval. At the same time his theory does provide a reasonable framework of thought which takes into account the effects of agonists, partial agonists and antagonists without introducing non-physical concepts like "efficacy" and "intrinsic activity".

My main criticism of Paton's important paper would be that, like most physiologists, he has confined his attention to what are commonly called excitatory effects. From a chemical point of view relaxation is as active a process as excitation. I would go so far as to say that all drug actions are

inhibitory, either interrupting a biochemical state which restrains an excitatory effect, or preventing a biochemical process which leads to excitation: thus nicotine in small doses may interfere with the biochemical mechanisms maintaining polarization of specialized cell membranes, so enabling depolarization (and therefore excitation) to occur, or in large doses it may either prevent the access of the natural chemical transmitter to its proper receptors or so alter the cell membrane that the natural chemical transmitter becomes ineffective.

If you agree with me that drugs may legitimately be defined as chemical substances *foreign* to the animal economy, it is easier to think of them as blocking normal biochemical mechanisms than as initiating physiological events. It is partly a matter of probability. We know that the resting state of any organ is maintained by an active biochemical mechanism and that physiological events are the result of a complex succession of biochemical reactions. It seems to me highly improbable that a chemical substance foreign to this elaborate biochemical machinery can do more than interfere with it, blocking some essential biochemical state or reaction which controls or directs some physiological event.

Moreover if we, as chemists, accept this point of view, our task is simplified. Directly we encounter a new drug with interesting properties, our first question will be: how does the new drug interfere with normal biochemical states or reactions?

It must be admitted that a few drugs can imitate the effects of normal chemical transmitters or hormones; also that some mutagenic drugs, by changing the chemical nature of normal cell constituents, may initiate unusual chemical reactions; but I am convinced that the vast majority of drugs act by interfering with normal biochemical mechanisms.

The fourth method of investigating the chemical aspects of drug action is, of course, the classical method of trying to make sense of structure-action relationships, particularly with the sharp changes in potency or in the types of activity resulting from relatively small changes in chemical structure. It would be impossible for me to deal with all aspects of this problem in the time at my disposal; all I can do is to deal with a few general considerations. First, the general problem of structure-action relationships is complicated by various factors such as the ease of access of the drug to its main sites of action, the metabolic hazards which it must encounter before it reaches its site of action and the rate of its metabolic destruction and excretion after it has reached its site of action.

Let us consider first three classes of drugs which almost certainly owe their therapeutic effect to true chemical combination with tissue constituents.

There is little doubt that all arsenical drugs act by the formation of thioarsonites with enzymes that depend for their action upon the integrity of sulphhydryl groups; yet even if we compare arsenoxides of closely related structure, not all are equally effective: *e.g.* in the homologous series 4-OAsC<sub>6</sub>H<sub>4</sub>(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H, butarsen (*n* = 3) is outstanding in trypanocidal activity *in vitro* whereas it displays no exceptional toxicity in mice. It seems likely that butarsen penetrates the cell membrane of trypanosomes more easily than its lower and higher homologues<sup>12</sup>.

Similarly, dialkylphosphofluoridates are not equally toxic and this appears

to be due to differences in the ease of hydrolysis of dialkylphosphorylcholinesterases, the di-isopropyl compounds being less easily hydrolysed than the methyl and ethyl compounds.

Again, tertiary  $\beta$ -chloroethylamine drugs of the dibenamine type differ in their potencies although it seems likely that they all act by alkylating some unknown constituent at sympathetic nerve endings. The high activity of dibenylamine is particularly interesting, because the  $\alpha$ -methyl- $\beta$ -phenoxyethyl group is probably a haptophoric group; thus  $\beta$ -phenoxyethyl benzylethylamines, which lack the  $\beta$ -chloroethyl group, display anti-adrenaline properties, and, therefore, have an affinity for sympathetic nerve endings.

When we consider drugs which are unlikely to form true covalent compounds with tissue constituents—and these are the vast majority of drugs—we ought to expect activity to depend upon factors like molecular dimensions and shape, ionic, polar or hydrogen-bonding groups, simply because attachment of the drug molecule to a receptor will depend upon a combination of relatively weak forces.

The stereospecificity of many drugs is the easiest example to consider, since it implies a three-point attachment to an asymmetric molecule in living tissues. It is worth noting in this connexion that stereospecificity is displayed by drugs which antagonize the effects of non-asymmetric substances like acetylcholine and histamine, so that the antagonist is probably forming a complex with groups other than those directly involved by the agonist, as well as with the agonist receptor itself.

The effect of molecular dimensions and shape is well illustrated by Albert's work on bacteriostatic acridine compounds<sup>13</sup>. Of the five possible aminoacridines only two (2 and 5-aminoacridine) were strongly bacteriostatic; they were both strong bases, but 4-aminoquinoline, an equally strong base, was inactive. However, fusion of a third aryl nucleus as in 5:6-, 6:7 or 7:8-benzo-4-aminoquinolines restored bacteriostatic activity. Similarly, 1:2:3:4-tetrahydro-5-aminoacridine was not bacteriostatic but fusion of another aryl nucleus at the 6:7, 7:8, or 8:9 positions restored activity. Albert's results indicated, therefore, that a minimal flat area (3 aryl nuclei) was as essential a factor as more-or-less complete ionization of the base at physiological pH values.

I could go on giving examples of how spatial and dimensional factors affect drug action, as we should expect them to do if drug-receptor complexes depend upon a multiplicity of weak forces. A necessary consequence of this way of looking at drug-receptor interactions is that the drug molecule must fit the receptor in spatial terms. A good example would be the spatial relationships of bis-onium salts as ganglion blocking agents. Gill<sup>14</sup> has shown that both in the methonium series and in the *para*-oniumphenylalkylonium series, maximal ganglion-blocking activity occurs for compounds in which the cationic groups are about 6–7.8 Å apart.

Again analogues of acetylcholine are particularly sensitive to the alkylation of the onium *N*-atom: *e.g.* in acetylcholine, replacement of more than one *N*-methyl group by ethyl almost abolishes parasympathomimetic properties<sup>15</sup>, whereas in benzilic esters of choline, replacement of one *N*-methyl group by ethyl gives maximal atropine-like properties<sup>16</sup>.

All these examples, and many others that I have no time to mention, force us to think in terms of an exact fit between the drug and the receptor.

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