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The drug-receptor complex

A. S. V. BURGEN

The rate of association of drug and receptor when limited by diffusion alone will correspond to a basic rate of $2{\cdot}5 \times 10^9$ litres/mole sec and will have a net activation energy of 3–4 kcal/mole. This rate will not be significantly increased by attractive forces between drug and receptor but could be reduced by repulsive forces. It could be considerably reduced by the presence of bound water and ions, by the requirement of activation energy for combination and by geometrically restricted access to the receptor. Complex formation is due to the stabilisation of the drug in the force field from which it can escape only on acquisition of kinetic energy greater than the potential energy of the field; the fraction of molecules acquiring this threshold kinetic energy can be calculated from the Boltzman equation. The rate of dissociation of the complex is the rate of loss by free diffusion multiplied by the Boltzman factor. The lifetime of drug receptor complexes is long enough to enable molecules undergoing collision in the non-ideal aspect or conformation time to present in the ideal state.

CONTEMPORARY ideas of drug action and drug specificity are all based on the assumption that the initial process in drug action is the formation of a reversible complex* between the drug and a cell component generally known as the drug receptor. The idea of a specific drug-receptor complex originated with Langley and Ehrlich and was developed into a quantitative theory by Langmuir and Clark. Until comparatively recently this general basis was accepted but little work was undertaken to evaluate it critically. The seeds of doubt were sown by the realization by Stephenson (1956) that there was no necessary reason to equate the maximum physiological response of a tissue with maximal occupancy of the receptors. Obviously the reaction of the drug with the receptors can be the limiting factor, but equally other factors in the train of events leading from drug combination to the physiological response, e.g., myofibril contraction, might be determining the maximum response and influencing the slope of the dose response curve. The possibility that spare receptors exist throws into doubt much of the previous work and it is regrettable that we still lack an unequivocal tool for assessing the magnitude of receptor occupancy by agonists. However, the single most potent factor responsible for the present revival of interest in drug-receptor interactions has been the work of Paton (1961), who has questioned whether agonist action is a direct function of drug-receptor complex concentration and has produced evidence in favour of the view that it is the act of formation of the complex that is important, i.e. that agonist action is a function of the turnover of drug-receptor complexes.

This approach has naturally focussed attention on kinetic aspects of the drug receptor complex. In this paper an attempt is made to define a theoretical basis for drug-receptor kinetics based on molecular theory.

From the Department of Pharmacology, University of Cambridge, Downing Street, Cambridge.

* Even in the relatively few cases where the drug ultimately links on to the receptor through a covalent bond it is likely that an initial reversible complex is formed; a comparable situation is the Michaelis complex of an enzyme and substrate.

Complex formation

Reaction theory postulates that for two molecular species to react it is first necessary for them to approach to a collision radius at which distance intermolecular forces can act. We shall see later that intermolecular forces are of short range and diminish to negligible values when the internuclear distance of the reactants is greater than about 12 Å, i.e. they operate over 1–2 molecular diameters.

If the reactants come within a collision radius they have a probability of reacting which may vary from 1 to 0.

We can define the limiting rate of reaction as that due to transport of the reactants into a collision radius by diffusion, any diminution below this rate must be accounted for by steric or energetic barriers to complex formation. An increasing number of reactions, particularly of an ionic type, have been shown by a variety of techniques for measuring fast reactions to be diffusion limited; examples are the combination of iodine atoms to form an iodine molecule or the quenching of the fluorescence of uranin by halide ions (Caldin, 1964). By contrast ordinary chemical reactions are slower by several orders of magnitude.

The mathematical theory of diffusion limited reactions laid by Smoluchowski (1917) (see also Alberty & Hammes, 1958; Noyes, 1961; Caldin, 1964) was concerned with the kinetics of coagulation of colloids by electrolytes.

His formulation of the problem was to calculate the rate at which particles diffuse into a hemispherical cavity surrounding the target site.

The result obtained is

$$n = \frac{4\pi r_0 D_{12} NC}{1{,}000} \qquad (1)$$

Since the flux rate $n = k_1 C$, where k_1 is the collision rate constant, then

$$k_1 = \frac{4\pi r_0 D_{12} N}{1{,}000} \qquad (2)$$

(r_0 = radius of target molecule; D_{12} = relative diffusion constant of the reactants; N = Avogadro's number, i.e. $6{\cdot}02 \times 10^{23}$ molecules/mole).

In dealing with the reaction between a mobile drug and a structurally fixed receptor D_{12} becomes the simple free diffusion constant of the drug. It is a convenience to eliminate diffusion constants from the equation completely by using the Stokes–Einstein equation

$$D = \frac{kT}{6\pi\eta \cdot r_D} \qquad (3)$$

(k = Boltzman's constant; T = absolute temperatures; η = viscosity of the medium; r_D = radius of diffusing molecule).

Substituting (3) in (2) we obtain

$$k_1 = \frac{2RT}{3{,}000\eta} \cdot \frac{r_0}{r_D} \qquad (4)$$

(R = universal gas constant).

In the case of the majority of drug-receptor interactions it can be assumed that $r_o \approx r_D$ since there is a spatial correspondence of the drug and its complementary site.*

For this case $k_1 = 2{\cdot}5 \times 10^9$ litres/mole sec (37° C).

This simple equation gives values in close agreement with the experimental measurements of diffusion limited reactions and its theoretical basis appears to be justified.

The universal dependence of the rate constant on viscosity is of considerable interest and accounts for most of the temperature dependence of collision rate. Since the viscosity of dilute aqueous solutions decreases by about 20% for each 10° C rise in temperature we can expect the rate constant to increase by 25–30% for a corresponding rise in temperature. In chemical kinetics changes in rate with temperature are usually evaluated by the Arrhenius equation

$$k_1 = Ae^{-E_a/RT} \quad .. \quad .. \quad .. \quad (5)$$

where E_a is an energy of activation; the activation energy of viscosity is 3–4 kcal/mole. This energy is attributable to the cage effect, i.e. each solute molecule is effectively enclosed by solvent molecules and to become translocated must bypass a solvent molecule. The probability of the molecule doing so depends on its kinetic energy. This is the minimum activation energy associated with molecular complex formation† and only when the activation energy exceeds this value can we presume that other steps requiring activation are present. The estimation of activation energy and viscosity dependence are clearly important tools in evaluating diffusion limited reactions but a word of caution is necessary. The viscosity of solutions can be increased most readily by the addition of high polymers such as proteins, dextran, or polyvinylpyrrolidine, but it must not be expected that these substances will materially change the rate of diffusion limited reaction because the viscosity enhancing effect of these solutes is due to frictional effects between the macromolecules that have little effect on the microscopic viscosity of the solvent.

The formation of the drug-receptor complex depends on the existence of a measure of complementarity between the two structures so that potential sources of intermolecular force can co-operate to form the complex. These forces, electrostatic, dispersion and hydrophobic, are all extensive, so that there is a force-field normal to the receptor surface acting upon the drug molecules diffusing in the neighbourhood. This field will modify the rate of bombardment of the receptor and will increase the rate if the net force is attractive and decrease it if the net force is repulsive. This effect can be incorporated (the second term in the bracket) in the Fick diffusional equation (Debye, 1942).

$$\frac{dn}{dt} = DA\left[\frac{dc}{dz} + \frac{c}{kT}\frac{dU}{dz}\right] \quad .. \quad .. \quad .. \quad (6)$$

* For large drugs such as polypeptides this may not be true if only a part of the molecule is directly concerned in complex formation.

† In a few special cases the activation energy is less than 3–4 kcal/mole when chain reactions or quantum mechanical tunnelling are involved.

where U is the free energy of the drug molecule at a distance r from the receptor.

Solution of this equation shows that the rate constant k_1 of the Smoluchowski equation (2) must be corrected by a factor f dependent on the solution of equation (7)

$$f = -\frac{1}{\int_{d_e}^{\infty} \frac{e^{U/RT}}{r^2} \cdot dr} \qquad \ldots \quad (7)$$

(d_e = separation of drug and receptor centres in the drug-receptor complex at equilibrium).

The numerical value of f will depend on two factors, firstly the way in which U varies with distance of separation of drug and receptor and, secondly, on the absolute value of U at the equilibrium distance. The dependence of most intermolecular forces on distance can be expressed by a simple inverse power, i.e.

$$U = ar^{-p} \qquad \ldots \quad (8)$$

For ionic forces p = 3 due to the operation of dielectric polarisation and shielding by the ionic atmosphere, for dipolar forces p = 3–6, for dispersion forces p = 6 (recent work has given evidence of both shorter and larger range dispersion forces for which p = 4–8) and for van der Waals' repulsive forces p = 9–12. There is no clear understanding as yet of the distance dependence of hydrophobic interaction, but simple geometric considerations suggest p = 2–4. Fig. 1 shows the dependence of f on U for values of p = 3 and p = 9.

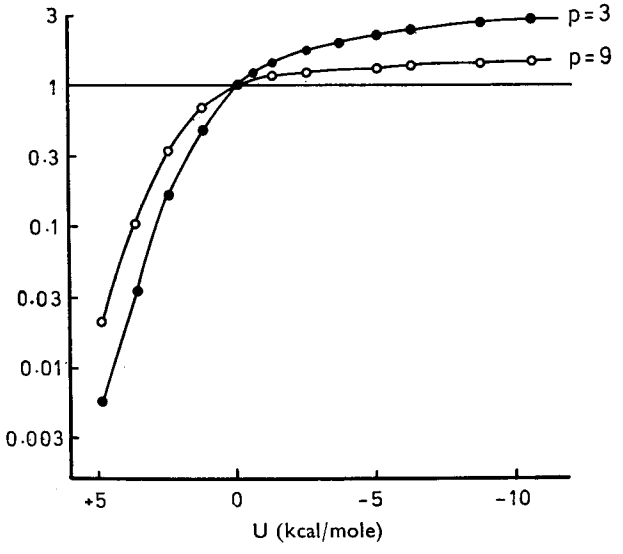

FIG. 1. The modifying effect of the force field on the rate of collision. The curves are calculated for forces varying as r^{-3} and r^{-9} respectively; intermediate values of p give intermediate values for f. The curves calculated by computer from equation (7). The ordinate gives the values for the free energy of complex formation at equilibrium.

It can be seen that the attractive force field makes a small but significant contribution to the rate of collision. For instance, in a drug interaction with the receptor whose free energy is -10 kcal/mole (equivalent to pA or pI = 7·0) in which half the interaction can be attributed to third power and half to sixth power forces, the collision rate will be increased by a factor of 2·3. On the other hand, repulsive forces can have a much more significant retarding effect. For instance, an ionic repulsive force (e.g. between groups in drug and receptor of like sign) whose magnitude at equilibrium is $+5$ kcal/mole will reduce the rate of collision by over 100-fold.

Since the net interaction between a drug and its receptor depends on the summation of fractional attractive forces of specific and complementary features of drug and receptor site, these will be fully realized only when the drug is lying in a unique rotational plane. Only if the drug molecule is presented in this optimal aspect during the approach to the receptor will the full accelerative effect of the force field be attained. In all other aspects the effect of the force field will be less and the acceleration correspondingly diminished. Our next problem is to estimate the probability of the optimal aspect of the drug being presented during approach.

Einstein, in considering Brownian motion of particles showed that the rotation of a spherical particle in solution is given (see Pollard, 1962) by

$$\bar{\theta}^2 = \frac{kTt}{4\pi\eta r^3} \qquad (9)$$

This equation for rotational diffusion may be compared with the equation for translational diffusion

$$\bar{x}^2 = \frac{kTt}{3\pi\eta r} \qquad (10)$$

Combining the two equations we find

$$\theta = \sqrt{\tfrac{3}{4}} \cdot \frac{x}{r} \qquad (11)$$

where θ is the rotation in radians and x/r, are equivalent units (Å). If a molecule is to display all aspects it must rotate through 2π radians so that the probability of displaying all aspects is

$$P = 0{\cdot}86\,\frac{x}{2\pi r} \qquad (12)$$

Now let us define as a significant acceleration of diffusion an increase of 50% over simple diffusion. The data of Fig. 1 shows this is attained at $U = -1{\cdot}4$ kcal/mole for an r^{-3} force and $U = -4{\cdot}7$ kcal/mole for an r^{-6} force. If we take the equilibrium distance to be 5 Å and $U_e = -10$ kcal/mole, we may calculate the available distance over which acceleration operates as 4·6 Å and 0·68 Å respectively and the corresponding values of P are 0·16 and 0·023 (drug radius assumed to be 4 Å). The low values of P show that the accelerative force is negligible for r^{-6} forces and of little importance even for the more extensive r^{-3} force. The exception to this rule will be in drugs showing a high degree of symmetry

(for instance, tetramethylammonium). Actually the values for P will be effectively reduced still further for the majority of drugs because they are not rigid molecules but can exist in several conformations determined by hindered rotation about valence bonds. Since only one of the set of conformations will be able to interact maximally with the force field, the overall probability must include corrections for both rotation and conformational effects.

We will assume at this point in our argument that the arrival of a drug at the receptor in an unsuitable aspect or conformation does not necessarily prevent a complex being formed although it may reduce the probability of its being formed; we will reserve until later a discussion of this effect.

The theory of drug antagonism states that if a receptor is already occupied by a drug, the complex cannot combine with another molecule. While this is not necessarily true we will assume it to be true in the next stage of our argument. We can state our rule as follows: complex formation only occurs between naked drug and receptor molecules. If the drug or receptor sites have an affinity for some ubiquitous component of biological solutions then a proportion of drug molecules and receptor sites will already be occupied and so be unable to react with each other. The obvious substances to consider are the inorganic ions and water molecules.

Consider first the case where a drug is a cation and there is a complementary anionic site on the receptor. If the anionic site is occupied by an alkali metal such as sodium, the approach of the drug will be sterically hindered and in addition the positive charge of the sodium ion will cause electrostatic repulsion. In principle, the problem is a classical one in the theory of electrolytes, i.e. the problem of ion pair formation. Bjerrum (1949) showed that for small univalent ions of radius 2 Å the degree of association is less than 10% in an ambient electrolyte concentration of 0·15 M. The same kind of argument will apply to drug molecules except that because of the larger size of the cation the potential for forming pairs is reduced. Unfortunately, we cannot be so sure about the receptor. Let us assume for the moment that the receptor is a protein. The factors determining ion binding to proteins are imperfectly understood and are certainly more complex than for simple ions. Alkali metals have little or no affinity for most proteins (for example, plasma proteins), but there are exceptions and some enzymes for instance bind alkali metals rather strongly (Steinhardt & Beychok, 1964). The existence of the metal transferring proteins, transferrin and caeruloplasmin, point to a special affinity of these proteins for the polyvalent ferric and cupric ions. We obviously cannot make a rule about cation binding. Furthermore, it is common to find that isoelectric proteins bind anions (such as chloride) more strongly than cations and that the differences in binding of different anions is quite striking. The likely explanation is that ion binding by proteins is not due solely to Coulomb attraction but is also influenced by the polarisability of both the ions and the protein. It is just not possible at present to establish any general rule for ion binding and we

must recognise that this could be a significant effect in reducing the frequency of complex formation. However, since the local peculiarities are likely to be restricted to the receptor, ion occupancy should have a uniform effect on the whole series of drugs combining with the receptor and which interact with the ionic site.

THE EFFECT OF HYDRATION

The problem of water binding is even more difficult to evaluate. We know that proteins bind quite large amounts of water of the order of several molecules per residue and there is no doubt that water is bound on drugs too. Binding by hydrogen bonding sites is the most obvious mechanism but attraction by other dipolar forces must also occur. There is also little doubt that water is eliminated when some aggregates form. For instance, water displacement has been studied quantitatively in the aggregation of collagen monomers. Indeed, it is more accurate to write the overall reaction between a drug and receptor in the form shown in equation (13).

$$\begin{array}{l} D \cdot mH_2O \rightleftharpoons D \cdot nH_2O + (m - n)\,H_2O \\ \qquad\qquad\qquad\qquad \searrow \\ \qquad\qquad\qquad\qquad\quad R \cdot D \cdot (n + y)\,H_2O \\ \qquad\qquad\qquad\qquad \swarrow \\ R \cdot xH_2O \rightleftharpoons R \cdot yH_2O + (x - y)\,H_2O \end{array} \quad \ldots \quad (13)$$

In general, we can expect the number of water molecules involved in hindrance of complex formation to be a function of both the total area of molecular interaction and also to be dependent on groups with a particular affinity for water molecules.

The probability of both drug and receptor sites being optimally hydrated at the time of collision is

$$P = (1 - \pi)^{\frac{(m + x) - (n + y)}{\pi}} \quad \ldots \quad \ldots \quad \ldots \quad (14)$$

(where π is the probability of finding an average binding site occupied by a water molecule).

Let us assume, for example, that $\pi = 0{\cdot}5$ then if

$$(m + x) - (n + y) = 4,\ P = 0{\cdot}004 \text{ and if}$$

$$(m + x) - (n + y) = 8,\ P = 0{\cdot}000015.$$

It is obvious that interference by hydration can be a very serious problem.

It may help to consider the operation of the hydration effect in another way. Suppose the interaction of two groups forming the complex is by dispersion forces, the interposition of a water molecule with a diameter of about 1·9 Å will reduce the free energy of interaction to about one tenth (ignoring the possibility of three body interactions and the malpositioning effect on other group interactions).

The effect of hydration will obviously be mitigated if despite the presence of bound water a weak complex can be formed with a lifetime which is large compared with that of water binding. This possibility will be considered later.

Koshland (1959) has suggested that where substrates and inhibitors combine with enzymes, the complex may not be the result of combination between the reactants in their initial state but that it may be induced by intermolecular forces. Evidence in support of this has been found in a number of cases, and the idea is obviously applicable to drug-receptor complexes and accords with the most widely held theory of agonist action, i.e. that agonists are capable of inducing conformation changes in the receptors. If such an effect occurs it will reduce the rate of complex formation below that for diffusion limited reactions according to the activation energy of the induced change and may be evaluated by equation (5). The presence of induced fit will be improbable if the overall activation energy is not greater than that expected for diffusion alone.

ACCESS TO THE RECEPTOR

Finally, we must consider the problem of geometrically hindered access to the receptor. Suppose that the receptor lies at the bottom of a crevice on the cell membrane. Diffusional access to the site may be severely hindered and in particular it may be impossible for malpresented molecules or molecules in the inappropriate rotational conformation to gain access. It is possible to set up model situations and evaluate the reduced frequency of complex formation on the theory of rotational diffusion outlined above.

The dynamics of the drug-receptor complex

It is usual to regard the total interaction of complex molecules with each other to be the algebraic sum of the interactions of the molecular subentities. Linear combination of forces has had a striking success in molecular physics despite the implicit simplification and this is an adequate reason for continuing this approach. The range of forces operating in molecular interactions include ion-ion, ion-dipole, dipole-dipole, hydrogen bonds (which may be regarded either as a variety of dipole-dipole force or of charge-transfer complex as Mulliken & Person, 1962, have suggested), dispersion forces and hydrophobic forces* and van der Waals' repulsion. Since detailed discussions of these forces have been provided recently by Webb (1963) and Gill (1965) it is only necessary to consider here some special aspects of these forces.

Dispersion forces had their origin in studies of gas reactions and particularly in the consideration of the thermodynamic properties of the inert gases and there have long been misgivings about their magnitude in liquids. McLachlan (1965) has recently been able to calculate their magnitude in liquids by a rigorous method and similar calculations have been made by Kestner & Sinanoglu (1965). The conclusion is that these forces are little reduced by solvent. The latter authors estimate that the interaction in water is 70–85% of that in the gas phase. The view that these forces are solely related to distance as r^{-6} has also been modified as evidence of r^{-4} and r^{-8} terms has been found (Buckingham, 1965);

* Dipole-induced dipole forces are too feeble to contribute appreciably to drug interactions.

the dispersion forces are therefore not necessarily as short range as was previously believed. The nature of hydrophobic forces is due for some revision because of spectroscopic evidence by Stevenson (1965) which demonstrates that liquid water contains very little monomeric water: this necessitates a revision of the thermodynamic basis of the hydrophobic bond presented by Marchi & Eyring (1964) and by Nemethy & Scheraga (1962). This also seems to lead to a reassessment of the role of molecular association through hydrogen bonds. Recently the role of hydrogen bonds has been minimised because of hasty assumptions of the competition of water hydrogen bonds with other intermolecular hydrogen bonds. This is at variance with the evidence for strong hydrogen bond participation in protein α-helix formation produced by Linderstrom-Lang and his collaborators. The virtual non-exchangeability of helix bonding hydrogens with deuterium does not agree with the postulation of the weakness of these bonds and of active competition by solvent.

While these facts must be taken into account in assessment of the binding capacities of different parts of drug molecules with the receptor, they need not concern us in developing the theory of the drug-receptor complex and its dissociation. All the forces mentioned above are extensive in space and with certain exceptions constitute a family of attractive forces obeying equation (8) in which the value of $p = 3–8$. The universal repulsive force is that due to invasion of the van der Waals' envelope of atoms and for which p has a value of 9–12; in some interactions, presumably not in strong ones, repulsion may also be due to like sign ions and dipoles. The net result of all these forces can be represented by potential energy contours of the type illustrated in Fig. 2.

The minimum of the energy diagram corresponds to the most stable position of the drug in the composite force field of the drug and receptor and is referred to as the equilibrium position. Closer approach is restrained by the steeply rising repulsive field and loss is retarded by the attractive field.

It must not be imagined, however, that the drug is held immobile in the equilibrium position. The drug molecules are subject to thermal agitation and acquire kinetic energies distributed according to the Boltzman law.

The probability of finding a drug molecule at any particular locus within the force field can be calculated therefore from the Boltzman law and is shown in Fig. 3. The meaning of this can best be understood by considering the behaviour of a drug molecule found at $t = 0$ at the equilibrium distance. If the drug molecule acquires a kinetic energy U kcal/mole it will move away from the equilibrium position. For simplicity we will consider motion in a single plane, i.e. either towards or away from the receptor. The drug molecule will continue to move until its initial kinetic energy is balanced by the increased potential energy of its new location. It will then come to rest and the potential energy gradient will then return the molecule to the equilibrium distance (Fig. 3). The same process will apply whether the drug travels away from the receptor or towards it, although in the latter case it will travel a shorter

distance before coming to rest (for any value of the kinetic energy) because of the steeper rise of the field with distance on this side of the equilibrium position. A drug will be able to escape from the force field only if the kinetic energy is greater than the potential energy of the field (i.e. in the example shown in Figs 2–3 the kinetic energy must be greater than −10 kcal/mole). The frequency with which molecules exceed a given kinetic energy is given by the Boltzman equation

$$f = e^{U/RT} \quad .. \quad .. \quad .. \quad (15)$$

Inserting the value of U = −10 kcal/mole, we find that the probability of a molecule escaping is only 10^{-7} of its remaining in the field.

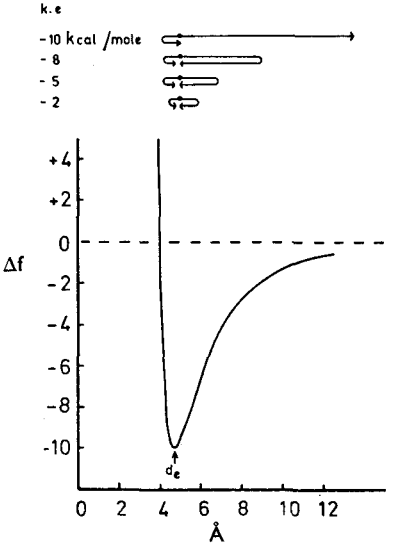

FIG. 2. Potential energy diagram for drug-receptor interaction. The curve has been calculated assuming an equilibrium distance between atomic centres of 5·0 Å and a free energy of interaction of −10 kcal/mole. The attractive forces have been divided equally among r^{-3} and r^{-6} forces, and the repulsive force has been assumed to vary as r^{-9}. Ordinate Δf in kcal/mole. The abscissa is the separation between the atomic centres (Å). In the upper part of the diagram there is shown in a schematic simplified way the behaviour of molecules initially at the equilibrium distance. The arrows indicate their behaviour on acquiring kinetic energy as indicated in the direction normal to the receptor surface.

This relationship is the main determinant of the inverse dependence of the strength of drug-receptor complexes and the velocity of their dissociation. The absolute rate of dissociation will be the free diffusion rate away from the receptor multiplied by this probability factor.

The relatively prolonged residence of drug molecules in the force field is the reason for not expecting the formation of effective complexes to be seriously reduced by collision in the wrong presentation or conformation. Provided that the free energy of interaction is strong enough in these cases to ensure that the molecule remains within the field long enough to rotate to the correct presentation or conformation it can then

develop the full potentialities of the field. An idea of the time required for presentation in all aspects may be derived from equation (9) and is about 4×10^{-9} sec. The period of rotation of hindered bonds is about 10^{-9}–10^{-11} sec. The lifetime of the complex for comparison may be evaluated as follows. Let the equilibrium free energy for the correct conformation and presentation be -10 kcal/mole and let us assume that in the incorrect approach this is reduced to -2 kcal/mole. If the rate of

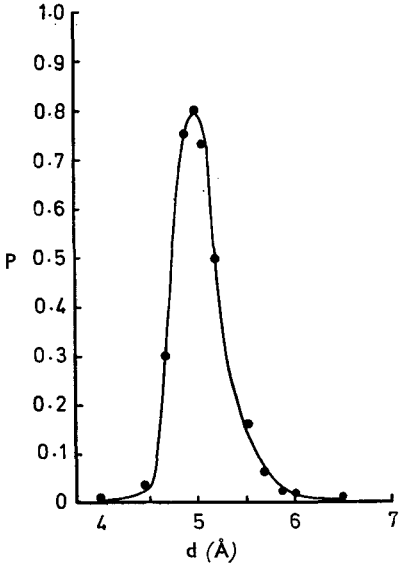

FIG. 3. From the potential energy diagram of Fig. 2 and the Boltzman distribution of energies [equation (15)] one can calculate the probability (P) of finding drug and receptor separated by a given distance (d) at any instant in the lifetime of the drug-receptor complex.

association is the maximum permitted by diffusion then the lifetime of the complex will be 10^{-8} sec; it is actually likely that the rate of association will be smaller than this (see Section 3) and the lifetime of the complex will be much longer. In any case the lifetime of the complex is long enough to reduce to minor proportions the effect of wrong presentation. An exception to this rule is likely to occur only when the receptor is buried in a crevice on the receptor surface or when the drug is very asymmetrical when adjustment of fit will be restricted.

The application of the theory of complexes to experimental results

It is unfortunate that very little work on the kinetics of drug receptor interactions or on molecular associations with proteins has been carried out so that this section must perforce be brief.

We will consider first the reaction between haptens and antibody to form complexes. Day, Sturtevant & Singer (1963) used a fast reaction technique to measure the kinetics of association of 2,4-dinitrophenyllysine

and an antibody against *dinitrophenylalbumin*. k_1 was found to be 8×10^7 litres/mole sec: similar rates were found with ϵ-dinitrophenyl-aminocaproate and dinitrophenylazo-1-naphthol 3,6-disulphonic acid. Similar rates have also been found by Froese, Sehon & Eigen (1962) and Froese & Sehon (1965) for nitrophenyl and phenyl arsonic haptens (Table 1). These values for association rates are between 1–10% of the maximum expected for a diffusion limited reaction. For the dinitrophenyl antibody the activation energy $E_a = 4{\cdot}1 \pm 1{\cdot}0$ kcal/mole, i.e. not significantly more than expected for a diffusion limited reaction. It is unlikely, therefore, that an induced fit operates in this case.

TABLE 1. KINETIC CONSTANTS FOR HAPTEN-ANTIBODY COMPLEXES

Antibody determinant	K litres/mole	k_1 litres/mole sec	k_2 sec^{-1}
2,4-Dinitrophenyl	$7{\cdot}3 \times 10^7$	8×10^7	1·1
4-Nitrophenyl	$2{\cdot}4 \times 10^5$	$1{\cdot}8 \times 10^8$	760
4-Phenylarsonic	4×10^5	2×10^7	50

The most reliable data for a drug receptor at present available are those of Paton (1961) and Paton & Rang (1965) obtained on the smooth muscle of guinea-pig ileum. These data, which were obtained both from studies of antagonism and by measurement of the uptake of tritiated antagonists, are summarised in Table 2. The association constants are smaller by another order of magnitude than for the haptens but are still

TABLE 2. KINETIC CONSTANTS FOR ANTAGONIST-MUSCARINIC RECEPTOR COMPLEXES

Antagonist	K litres/mole	k_1 litres/mole sec	k_2 sec^{-1}
Atropine	$0{\cdot}98 \times 10^9$	$1{\cdot}76 \times 10^8$	$1{\cdot}79 \times 10^{-3}$
N-Methylatropine	$2{\cdot}1 \times 10^9$	$3{\cdot}50 \times 10^8$	$1{\cdot}67 \times 10^{-3}$
Lachesine	$0{\cdot}70 \times 10^9$	$2{\cdot}60 \times 10^8$	$3{\cdot}73 \times 10^{-3}$

of an order expected for a diffusion limited reaction; it is regrettable that no data are available for the activation energy. The complexes formed between antagonist and the muscarinic receptor are exceptionally strong and have very low dissociation rates. Now the free diffusion rate away from a plane source into an infinite medium is given by

$$C = \frac{A}{(\pi Dt)^{\frac{1}{2}}} \cdot e^{-x^2/4Dt} \quad .. \quad .. \quad .. \quad (15)$$

We can calculate the half time for atropine to diffuse the distance of one molecular radius from the receptor by free diffusion; this is found to be 3×10^{-10} sec. The dissociation rate constant is therefore $2{\cdot}3 \times 10^9$ sec^{-1}. The actual rate constant should be this value multiplied by the Boltzman probability, in this case $1{\cdot}02 \times 10^{-9}$. The value of k_2 therefore equals 2·3 sec^{-1}. This is much higher than the experimental value. The reason for this discrepancy is to be found in the low association rate compared with the rate given by free diffusion. This low rate can be attributed most probably to either (*a*) the reduced number of effective collisions due to hydration or ion occupation effects or (*b*) geometrical restrictions to diffusion. In the first case the free energy of complex

formation is increased because it should be related to the effective concentration in the bulk solution rather than the actual concentration. This is equivalent to increasing the free energy of association by −4·5 kcal/mole. The Boltzman factor then becomes $7{\cdot}2 \times 10^{-13}$ and the value of $k_2 = 1{\cdot}65 \times 10^{-3}$ sec^{-1}, which is very close to the experimental value. The corresponding theoretical values for *N*-methylatropine and lachesine are $1{\cdot}41 \times 10^{-3}$ sec^{-1} and $3{\cdot}45 \times 10^{-3}$ sec^{-1}. The correspondence of these results is in no way remarkable but simply results from the circular argument which defines diffusion as limiting for both association and dissociation. Since the mathematical approach is slightly different in the two cases it is an internal check on their validity. The second approach, of geometrical hindrance, leads to the same result but for a slightly different reason. Clearly any geometrical hindrance to the diffusion of drug towards the receptor must apply equally to diffusion away from the receptor. The hindrance factor is equal to

$$\frac{k_1}{2{\cdot}5 \times 10^9}$$

and multiplying the value of the crude estimate of k_2 by this factor will give the corrected value of k_2.

The purpose of this paper has been to outline a theoretical basis for drug receptor kinetics and although it leaves a number of quantitative uncertainties unsettled some of these are potentially resolvable by experiment and it is the author's hope that the presence of a theoretical background will encourage further experimental work in this field.

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