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THE INTERACTION OF HUMAN HAEMOGLOBIN WITH ALLOSTERIC EFFECTORS AS A MODEL FOR DRUG-RECEPTOR INTERACTIONS

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1 The release of bound oxygen from oxyhaemoglobin by allosteric effectors is considered as a model for those drug-receptor interactions where the primary response to agonist binding is the release of a second messenger species.

2 A theory of haemoglobin oxygenation, based on the two-state model of Monod, Wyman & Changeux (1965) is used to predict the relationship between 'pharmacological' response and dose of agonist. This relationship is the same as that derived from classical pharmacological occupancy theory.

3 The potency of an agonist is a weighted average of its affinities for the two conformational states of the receptor.

4 The efficacy of an agonist depends not only upon its preferential binding to one of the two conformational states, but also on its ability to alter the functional properties of that state by lowering the affinity of the state for the second messenger.

5 2,3-Diphosphoglycerate and adenosine triphosphate are approximately equipotent and of similar efficacy, but inositol hexaphosphate is about 500 times more potent and has a higher efficacy.

Introduction

The interaction of human haemoglobin with the natural effector substance 2,3-diphosphoglycerate (DPG) exhibits many of the properties of a classical drug-receptor interaction (Beddell, Goodford, Norrington, Wilkinson & Wootton, 1976; Goodford, 1977). If DPG is considered as the drug and haemoglobin the receptor, the biological response is the enhanced oxygen release at the partial pressure (P_{O_2}) of the tissues. This system may therefore be regarded as a model for a drug-receptor interaction where the primary response to agonist binding is the release of a second messenger bound to the receptor.

In fact, the sequence of events between the initial drug-receptor interaction and the observed biological response for classical pharmacological receptors such as the cholinoceptor is unknown, and the primary consequences of agonist binding are still a matter of conjecture. One current theory (Rang, 1974) is that agonist binding causes a conformational change of the membrane cholinoceptor from a 'closed' to an 'open' state resulting in an increased flux of metal ions across the cell membrane. Colquhoun (1973) and Thron (1973) following the lead of Karlin (1967) and of Changeux, Thiéry, Tung & Kittel (1967) have developed the two conformation theory for allosteric interactions in proteins due to Monod, Wyman & Changeux (1965) along such lines. On the other hand, studies on isolated acetylcholine receptor preparations have demonstrated a linkage between agonist binding and Ca^{2+} binding (Chang & Neumann, 1976; Rübsamen, Hess, Eldefrawi & Eldefrawi, 1976), and the possibility that the binding of an agonist to its receptor *in situ* causes the release of receptor bound Ca^{2+} as an initial event has been considered (Chang & Triggle, 1973; Rübsamen, Montgomery, Hess, Eldefrawi & Eldefrawi, 1976). These two interpretations are not necessarily incompatible since the opening of an ion channel could be dependent upon the release of calcium.

Unlike classical drug-receptor systems, haemoglobin may be obtained as a pure protein with no loss of function and the observed response (i.e., oxygen release) is a direct consequence of agonist (e.g., DPG) binding. Studies on the pharmacological interpretation of the haemoglobin/DPG interaction may therefore throw some light on classical drug-receptor systems which are experimentally more intractable. In the present paper the actions of the allosteric effectors DPG, adenosine triphosphate (ATP) and inositol

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hexaphosphate (IHP) on the oxygen dissociation curve of haemoglobin are interpreted as agonist effects from the point of view of the drug-receptor interaction.

Methods

Oxygen dissociation curve measurements

Oxygen dissociation curves were measured by a spectrophotometric method described previously (Beddell *et al.*, 1976; Goodford, St-Louis & Wootton, 1978) using human haemoglobin of low phosphate content (Paterson, Eagles, Young & Beddell, 1976). The solutions were 0.265 mmol dm^{-3} in haem, 0.05 mol dm^{-3} in HEPES buffer and 0.035 mol dm^{-3} in NaCl with varying concentrations of DPG, ATP or IHP. Measurements were taken at 37°C and the pH of the solutions was 7.3.

Free 2,3-diphosphoglyceric acid was prepared by ion exchange chromatography on Amberlite IR-120(H) from the pentacyclohexylammonium salt (Calbiochem A-grade) and converted to the potassium salt by titration to pH 7.3 with KOH. ATP was purchased as the disodium salt from Boehringer Mannheim and IHP was obtained from BDH as the sodium salt. Stock solutions of these effectors were prepared with a concentration of 0.1 mol dm^{-3} and were checked for phosphate content by the method of Ames & Dubin (1960) and stored at −20°C.

Curve fitting

Theoretical equations were fitted to the experimental points on the oxygen dissociation curves by a least squares minimisation procedure described previously (Powell, 1970; Goodford *et al.*, 1978). The function minimised was the sum of squares of the unweighted differences between measured and calculated saturation values. This method was also used to fit theoretical curves to the dose-response data, and in this case the sum of squares of the unweighted differences between measured and calculated responses was minimised.

Theory

It is generally agreed that the two-state model for allosteric proteins developed by Monod *et al.* (1965) provides the most appropriate simple basis for a theory of haemoglobin oxygenation. On this model the haemoglobin tetramer exists in an equilibrium between two quaternary conformational states, the deoxy (T) state with a low affinity (K_T) for oxygen and the oxy (R) state with a high affinity (K_R) for oxygen. If the equilibrium ratio between the R and T states in the absence of ligand is denoted by L, then the saturation, Y, of the haemoglobin with oxygen at a partial pressure p is given by:

$$Y = \frac{\alpha(1+\alpha)^3 + Lc\alpha(1+c\alpha)^3}{(1+\alpha)^4 + L(1+c\alpha)^4} \quad (1)$$

where

$$\alpha = pK_R; \quad c = \frac{K_T}{K_R}; \quad L = \frac{T}{R}$$

According to the original Monod *et al.* (1965) model, allosteric effectors such as DPG bind preferentially to the deoxy state which increases the value of L and leads to the characteristic right shift of the oxygen dissociation curve (Benesch, Benesch & Yu, 1968). This interpretation has now been shown to be an oversimplification (Minton & Imai, 1974; Goodford, Norrington, Paterson & Wootton, 1977) since DPG not only increases the value of L but also lowers the value of K_T (and thus c). Perutz has outlined the mechanism by which the value of K_T may be altered in two recent reviews (Perutz, 1976; 1978). Basically, in the oxy state there are no constraints on oxygenation and the value of K_R is the average of the oxygen affinities of isolated α and β subunits. However, in the deoxy state, oxygenation is hindered due to salt bridges between subunits. These are broken by the tertiary structural changes which accompany oxygenation, and K_T is therefore lower than K_R. The value of K_T will then depend upon the number and strength of these salt bridges. Allosteric effectors such as DPG bind between subunits (Arnone, 1972), increase the number of salt bridges and further lower the value of K_T.

The oxygen saturation function, Y, in the presence of a concentration, d, of allosteric effector with an affinity, K_D, for the deoxy state and an affinity, K_O, for the oxy state may then be written (Goodford *et al.*, 1978):

$$Y = \frac{\alpha(1+\alpha)^3(1+dK_O) + Lc\alpha(1+c\alpha)^3 + Lb\alpha(1+b\alpha)^3\,dK_D}{(1+\alpha)^4(1+dK_O) + L(1+c\alpha)^4 + L(1+b\alpha)^4\,dK_D} \quad (2)$$

where b is the reduced value of c in the presence of the effector.

Typical saturation curves for haemoglobin 'stripped' of organic phosphates and in the presence of 5 mmol dm^{-3} DPG are shown in Figure 1. The ability of DPG to effect oxygen release may be quantitated from such curves by measuring the decrease in saturation caused by a given concentration of DPG at a fixed partial pressure of oxygen. This measure of oxygen release is regarded as the pharmacological response on the present model. The choice of partial

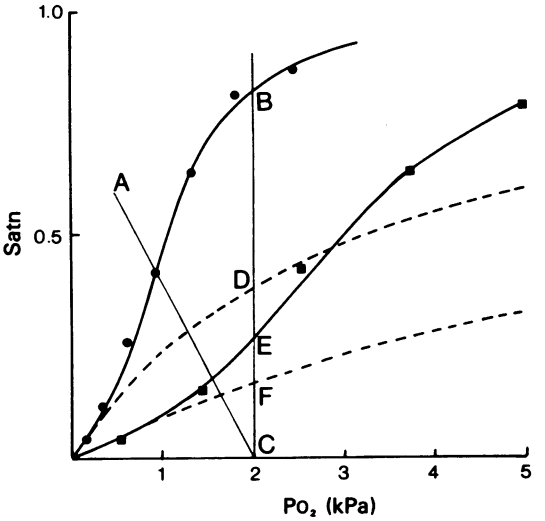

Figure 1 Typical oxygen dissociation curves for human haemoglobin in the absence (●) and presence (■) of 5.0 mmol dm^{-3} 2,3-diphosphoglycerate (DPG). Solutions were 0.265 mmol dm^{-3} in haem, 0.05 mol dm^{-3} in HEPES buffer and 0.035 mol dm^{-3} in NaCl at a temperature of 37°C and a pH of 7.3. The continuous curves were drawn using the best-fit parameter values obtained from fitting equation (1) to each set of points. The upper dashed curve is the theoretical hyperbolic saturation curve for an oxygen affinity of 0.312 kPa^{-1} (K_T for the stripped curve, see text) and the lower dashed curve is the theoretical hyperbolic saturation curve with an oxygen affinity of 0.100 kPa^{-1} (K_T for the 5 mmol dm^{-3} DPG curve).

pressure is somewhat arbitrary, but the present data have been analysed using a value of 2 kPa (given by the line BC in Figure 1) since this results in maximum discrimination between curves under the conditions of the experiments.

It is now necessary to derive an expression relating this response to the concentration of agonist (DPG). At constant pressure, the saturation expression (Equation 2) may be written:

$$Y = \frac{c_1 + c_2 d}{c_3 + c_4 d} \tag{3}$$

where c_1 through c_4 are constants and

$$c_1 = \alpha(1 + \alpha)^3 + Lc\alpha(1 + c\alpha)^3;$$
$$c_2 = \alpha(1 + \alpha)^3 K_O + Lb\alpha(1 + b\alpha)^3 K_D;$$
$$c_3 = (1 + \alpha)^4 + L(1 + c\alpha)^4;$$
$$c_4 = (1 + \alpha)^4 K_O + L(1 + b\alpha)^4 K_D$$

The response, S, is then

$$S = \frac{c_1}{c_3} - \frac{c_1 + c_2 d}{c_3 + c_4 d}$$

which may be rearranged to give:

$$S = \frac{eKd}{1 + Kd} \tag{4}$$

where

$$e = \frac{c_1}{c_3} - \frac{c_2}{c_4}; \quad K = \frac{c_4}{c_3}$$

Equation (4) is written in this form to illustrate its equivalence to the classical equation derived from occupancy theory of drug action (Stephenson, 1956). S is equivalent to Stephenson's stimulus, e to his efficacy and K to his association constant of the drug-receptor complex. For haemoglobin the observed response is equivalent to the stimulus, but for classical receptor systems this is not the case, although it is usually assumed that equal stimuli produce equal responses (Stephenson, 1956). In the present treatment efficacy and affinity may be related to the original parameters of the allosteric model giving:

$$e = \left\{\frac{\alpha(1 + \alpha)^3 + Lc\alpha(1 + c\alpha)^3}{(1 + \alpha)^4 + L(1 + c\alpha)^4}\right\} - \left\{\frac{\alpha(1 + \alpha)^3 K_O + Lb\alpha(1 + b\alpha)^3 K_D}{(1 + \alpha)^4 K_O + L(1 + b\alpha)^4 K_D}\right\} \tag{5}$$

$$K = \frac{(1 + \alpha)^4 K_O + L(1 + b\alpha)^4 K_D}{(1 + \alpha)^4 + L(1 + c\alpha)^4} \tag{6}$$

Unfortunately, these expressions do not simplify to any great extent, but they indicate the factors upon which efficacy and affinity may depend. Thus, according to the present theory the efficacy of an agonist depends on two factors. The primary determinant is the relative affinity ratio, K_D/K_O, of the agonist for the two conformations. Also important is the extent to which the binding of the agonist to the deoxy conformation lowers the value of K_T, which is given by the ratio b/c. In the special case of b/c = 1 and if $K_D/K_O \gg 1$ then a large concentration of agonist will effectively fix the haemoglobin in the deoxy conformation. The maximum efficacy obtainable would then be defined by the distance BD in Figure 1 from the stripped dissociation curve at B down to the upper dashed line, which is the hyperbolic saturation curve for haemoglobin molecules in the deoxy state with an oxygen affinity K_T. In practice, however, agonists such as DPG can actually shift the saturation curve below point D down to point E. Such an observation is shown in Figure 1, and it is this experimental finding which is the basis for rejecting the simple two-state model in favour of the interpretation having K_T reduced in the presence of DPG by the factor b/c. In this case (with $K_D/K_O \gg 1$) the hyperbolic saturation curve is shifted downwards to the lower dashed line in

Figure 1, and the maximum efficacy is increased substantially from BD to BF.

On Stephenson's interpretation the parameter K in equation (4), is the association constant of the drug-receptor complex. Equation (6) shows that on the present interpretation K is not a single affinity constant, but a function of the separate affinities K_D and K_O of the drug for the two forms of the receptor. For an agonist which does not lower K_T then b = c and equation (6) may be written:

$$K = \bar{R}K_O + \bar{T}K_D \qquad (7)$$

where

$$\bar{R} = \frac{(1 + \alpha)^4}{(1 + \alpha)^4 + L(1 + c\alpha)^4}$$

$$\bar{T} = \frac{L(1 + c\alpha)^4}{(1 + \alpha)^4 + L(1 + c\alpha)^4}$$

and $\bar{R}$ and $\bar{T}$ are the fractions of the receptor in the oxy and deoxy states respectively, in the *absence* of agonist. When $K_D \gg K_O$ the apparent affinity, K, will be given approximately by $\bar{T}K_D$, and if $\bar{T}$ is small at the pressure of measurement then K may be considerably lower than K_D. For an agonist which lowers K_T, b $\neq$ c and equation (7) no longer holds. In this case, for $K_D \gg K_O$ the apparent affinity will be lowered further, and will be given approximately by equation (8):

$$K = \bar{T}K_D \frac{(1 + b\alpha)^4}{(1 + c\alpha)^4} \qquad (8)$$

Results

Concentration-response curves for agonists

Figure 2 shows the log concentration-response curves for the three agonists of oxygen release DPG, ATP and IHP. Each point in this plot is derived from a set of seven observations on a single oxygen dissociation curve, measured at the relevant agonist concentration. The seven observations were least squares fitted with the equation of Adair (1925) to obtain continuous curves which were then used to calculate the differences in saturation levels at 2 kPa between the control curve and the curves in the presence of agonist. The Adair function was used since it resulted in good fits to the experimental points in all cases, even for the biphasic, flattened curves obtained when the concentration of agonist used was lower than the concentration of haemoglobin.

When the dose of agonist used was comparable with, or lower than, the haemoglobin concentration it was necessary to calculate the free solution concentrations of agonist at 2 kPa. These were estimated using the parameter values obtained by fitting the

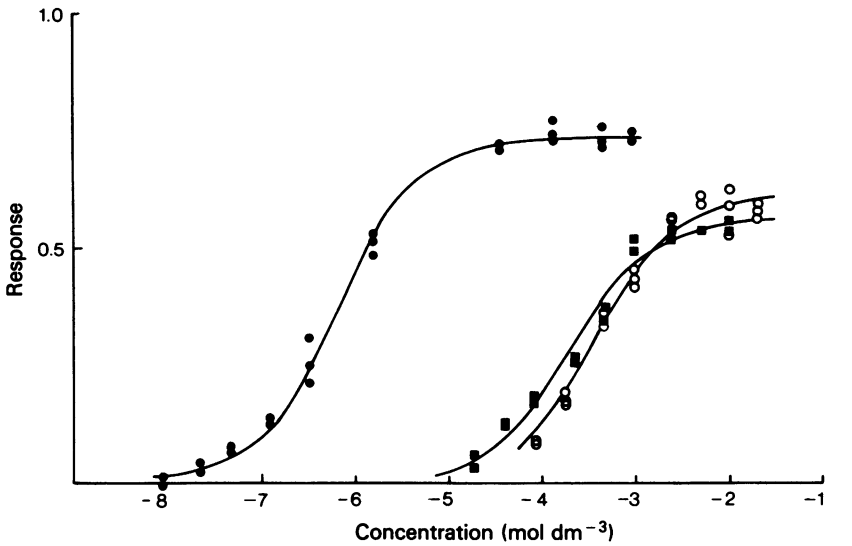

Figure 2 Log concentration-response curves for 2,3-diphosphoglycerate (DPG), adenosine triphosphate (ATP) and inositol hexaphosphate (IHP), where the response is the difference in saturation at 2 kPa between stripped haemoglobin and haemoglobin in the presence of the relevant concentration of agonist: (●) IHP, (■) DPG, (○) ATP. The responses were mostly measured in triplicate for ATP and IHP and in duplicate for DPG, giving a total of 23, 28 and 18 points for ATP, IHP and DPG respectively.

Table 1 Results of fitting equations (2) and (4) to the oxygen saturation data obtained in the presence of 2,3-diphosphoglycerate (DPG), adenosine triphosphate (ATP) and inositol hexaphosphate (IHP)

Agonist	e*	K* (mol^{-1})	K_D† (mol^{-1})	K_D/K_O†	b/c†	n_1‡	n_2‡
DPG	0.567 (±0.013)§	4800 (±500)	63,000 (±7000)	89	0.244	18	140
ATP	0.619 (±0.011)	2600 (±200)	40,000 (±4000)	138	0.289	23	182
IHP	0.737 (±0.006)	1,540,000 (±80,000)	38,500,000 (±4,500,000)	7900	0.163	28	217

* Best-fit values obtained by fitting equation (4) to the concentration-response data where the response is measured as the reduction in oxygen saturation at a partial pressure of 2 kPa in the presence of agonist.
† Best-fit values obtained by fitting equation (2) to the complete set of saturation data for each agonist.
‡ n_1 and n_2 are the numbers of points used in fitting the concentration-response curves and the saturation curves respectively.
§ Standard errors are given in parentheses, and were estimated from the matrix of partial derivatives obtained during the course of the fitting process.

three-state model of equation (2) to the complete set of dissociation curves for each effector as described by Goodford *et al.* (1978). This correction was particularly important for IHP, where at the lowest dose used (1 μmol dm^{-3}) the free solution concentration was estimated to be only 8.9 nmol dm^{-3}.

Equation (4) was least squares fitted to the experimental concentration-response curves so generated to give best-fit estimates of the asymptotic response, e, and the parameter, K, the apparent affinity for each agonist. The continuous curves in Figure 2 were plotted using these best-fit parameter values. In each case the experimental points are well-fitted by the theoretical curves confirming the expected theoretical relationship between the difference in saturation levels and the concentration of agonist given by equation (4). The best fit values of the parameters e and K are given in Table 1. Also shown are the K_D values, the K_D/K_O ratios and the b/c ratios obtained from fitting the three-state model of equation (2) to the complete set of saturation curves for each agonist (Goodford *et al.*, 1978). The results show that DPG and ATP have comparable apparent affinities as judged by the parameter K, but the affinity of IHP is about 500 times higher. Similarly DPG and ATP have comparable efficacies as shown by the asymptotic response, e, but IHP is appreciably more efficacious.

The higher apparent affinity of IHP is a result of its far higher affinity for the deoxy conformation of haemoglobin, K_D, compared to DPG and ATP. Equation (7) shows that when $K_D/K_O \gg 1$, which is the case for all three agonists, then the apparent affinity is determined largely by K_D. When b = c, the apparent affinity will be lower than K_D by the factor T, which is the fraction of haemoglobin in the deoxy state at 2 kPa in the absence of agonist. For the three agonists studied here, however, $b \neq c$, and the value of K will be still lower as shown by equation (8). As a result the affinities calculated from fitting equation (4) to the concentration-response data are all less than one tenth of the K_D values calculated by fitting the three-state model to the complete sets of saturation data (Goodford *et al.*, 1978). The efficacies of the three agonists depend on the ratios K_D/K_O and b/c (equation 5). IHP has a significantly higher efficacy than DPG or ATP since it has both a higher K_D/K_O ratio and a lower b/c ratio.

Discussion

In classical occupancy theory the observed response from a drug receptor interaction is a function of the saturation of receptor sites by the agonist substance (Stephenson, 1956). Thron (1973) and Colquhoun (1973) argued rather that the response depends on the fraction of receptors in the active or open conformation which is a different function of agonist concentration. However, the latter treatments (Thron, 1973; Colquhoun, 1973) could be made to give similar equations, for the most part, to those derived from classical theory. The approach used here, although specifically developed for the interaction of allosteric effectors with haemoglobin, shows that yet another mechanism of drug-receptor interaction will lead to the same predictions as the classical theory. In this case the pharmacological response is a function of the amount of a second messenger species bound at the receptor and this is indirectly modified by heterotropic allosteric interactions with the agonist.

In the present work we chose to measure the ability of an agonist to effect the release of a second mess-

enger as the differences in saturation of the receptor by this second messenger at a fixed concentration of second messenger. In fact, in a closed system the introduction of an agonist will result in a real increase in the free concentration of this second messenger, and to take differences in saturation levels at a constant concentration as the response is not strictly correct. The expression for the increase in the free concentration of second messenger as a function of agonist dose is complex (a quintic equation for the haemoglobin case) but it may be shown that the increases are given by the points of intersection of a line such as AC in Figure 1 with the measured saturation curves. This line meets the abscissa scale at the total concentration of second messenger (bound plus free), and has a slope which depends upon the relative concentrations of second messenger and receptor.

Under the conditions of the present experiments the line AC is steep (much steeper and closer to BC than it appears in Figure 1) and the relationship between the two measures of response is very nearly linear. However, this is not the case in general and at low second messenger concentrations (relative to the receptor) the relationship between the two responses may be markedly non-linear. In fact, under such circumstances the relationship between the true response and the concentration of agonist may deviate from the simple hyperbola, and may give slopes significantly less than unity when expressed in the form of a Hill plot. In spite of this complication however, the apparent affinity of an agonist is still given approximately by equation (6) and the relative efficacies for a series of agonists will be as predicted by equation (5). The use of differences in saturation level as response therefore seems justified, for the present experiments at least, since the factors which determine efficacy and affinity are much more readily interpreted on this model.

The equations used to derive the relationship between response and concentration of agonist (equation (4)) were based on the two-state theory of Monod *et al.* (1965), but in fact the same relationship would be obtained for any theory of haemoglobin oxygenation in which the interaction between haemoglobin and agonist is 1:1. In support of this there is good evidence for a single interaction site in deoxyhaemoglobin (Benesch, Benesch, Renthal & Gratzer, 1971; Arnone, 1972; Arnone & Perutz, 1974), but the binding to oxyhaemoglobin is much weaker and thus less well-defined. For the haemoglobin data it was therefore unnecessary to consider cases other than a 1:1 interaction between the agonist and each state of the receptor, and the data were well-fitted by the simple hyperbolic relationship between response and dose of agonist given by equation (4). On the other hand, many drug receptor systems show deviations from the hyperbolic relationship (Rang, 1974) which may be interpreted as arising from more than one binding site for the agonist and cooperativity in binding. This has also been seen in the binding of ligands to purified acetylcholine receptor preparations (Chang & Neumann, 1976; Gibson, 1976). In order to explain this type of behaviour it would therefore be necessary to extend the present model to consider multiple interaction sites for the agonist.

It is interesting to compare the predictions of the present model with those arising from the models of Colquhoun (1973) and Thron (1973), since the expressions for the efficacy and affinity of agonist substances are not the same. Thron (1973) used a complex definition of stimulus, S, shown in equation (9):

$$S = \left(\frac{L\bar{R}}{1-\bar{R}}\right)^{1/n} - 1 \qquad (9)$$

where n is the number of binding sites for the agonist. He then obtained an expression relating stimulus to the parameters of the allosteric model (Monod *et al.*, 1965) of the same form as equation (4). Efficacy was determined by the ratio of the affinities of the agonist for the two conformations, i.e., $e = K_T/K_R - 1$, where K_T and K_R are defined as the *dissociation* constants for agonist binding (Thron, 1973). Comparison of this expression with equation (5) is complicated by the different definitions used, but if the T (deoxy) state is regarded as the active or open conformation, and noting that *association* constants have been used in the present treatment, then the above expression becomes instead, $e = K_D/K_O - 1$. Thus, this expression predicts, as does equation (5), that efficacy depends on the ratio K_D/K_O, but suggests that there is no limit to its absolute magnitude, in agreement with Stephenson's (1956) empirical definition of efficacy. In contrast, equation (5) predicts a limited range of values for efficacy. For the special case in which the agonist does not alter the affinity, K_T, of the second messenger for the deoxy state of the receptor (b = c) then the limiting efficacy is defined by the distance BD in Figure 1. In addition, equation (5) allows for the agonist to lower the value of K_T, which has no analogy in the treatments of Colquhoun (1973) and Thron (1973). In this case, in the limit of $K_T = 0$, efficacy is further increased, but only to a maximum value defined by the distance BC which corresponds to complete displacement of the second messenger from the receptor.

The form of efficacy implied by the present model, i.e. with a limited range of values, is quite close to Arien's (1964) concept of intrinsic activity which varies from unity for a full agonist, to zero for an antagonist, with partial agonists having intermediate values. However, Stephenson's (1956) concept of efficacy has gained more universal acceptance since it provides a ready explanation for the apparent occurrence of spare receptors. Spare receptors are invoked

mainly to explain the fact that powerful agonists are still able to elicit a maximal response when a large proportion of the receptor molecules are apparently blocked by a slowly dissociating competitive antagonist or an irreversible antagonist. However, if it is assumed that agonists and antagonists occupy different receptor sites, as has been suggested by Ariens & Beld (1977), and that the competition is allosteric rather than direct, then the concept of spare receptors is no longer required in this context. The receptor site for the antagonist may be fully occupied, but the agonist site will still be available, and if K_D is much larger than K_O for the agonist then it will still be able to produce a virtually maximal response, although a much higher concentration may be needed.

The apparent affinity of an agonist according to the Thron (1973) model, is in most cases equivalent to the affinity of the agonist for the closed conformation of the receptor, which is given by K_O using the present definitions outlined above. On the present treatment the apparent affinity is a weighted average of the affinities of the agonist for the two conformations of the receptor (equation 7). For $K_D \gg K_O$, then apparent affinity is determined largely by K_D rather than K_O, but equation (8) shows that in fact the apparent affinity may be considerably lower than K_D, and in this case it is possible that in practice the measured affinity may be closer to K_O than K_D.

The actual magnitudes of apparent affinity and efficacy for a given agonist will of course be determined by the free concentration of second messenger in the absence of agonist. For the haemoglobin data a Po_2 of 2 kPa was used in analysing the results but inspection of the complete dissociation curves (Goodford *et al.*, 1978) indicates that although the results may be qualitatively similar at different Po_2 values, there may be large quantitative differences. For instance, at a Po_2 of 5 kPa, IHP will still have high efficacy, but the efficacy of DPG and ATP will be considerably reduced. At a Po_2 of 1 kPa, however, there will be much smaller differences between the efficacies of the three agonists. Such effects could provide an explanation for the different responses of receptors from different tissues since it is not unlikely that the resting level of second messenger may vary from tissue to tissue, even if the chemical structure of the receptors themselves is identical.

It should be emphasized that the present interpretation is only intended as a model for the initial event in a drug receptor interaction involving the release of a second messenger. The present data were obtained for a homogeneous solution under equilibrium conditions, whereas functional receptor systems may be heterogeneous due to phase boundaries, and dynamic events may play a crucial role in their function. Nevertheless, the final response is necessarily related to the initial interaction of the drug with the receptor protein, and it is important to delineate the factors which determine this interaction at the molecular level. The present approach allows efficacy and potency to be defined quantitatively in terms of physically meaningful parameters. It does away with the need to invoke spare receptors in certain cases, and provides an explanation for quantitative differences in the responses of the same receptor in different tissues. Moreover, it is open to detailed examination in appropriate purified receptor systems, and may therefore be used to design further experiments to improve our understanding of agonist receptor interactions.

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