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Bidirectional Collision Coupling in the Regulation of the Adenylate Cyclase

The Allozyme Hypothesis for Receptor Function

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SUMMARY

A mathematical analysis is presented of an extension of the collision coupling model for the regulation of the adenylate cyclase. The analysis assumes that the adenylate cyclase is switched on by a brief interaction with an agonist-occupied activating receptor. It remains on until it is switched off either by a brief interaction with an agonist-occupied deactivating receptor or with a postulated basal deactivating system. Only one species of agonist·receptor complex accumulates, so that agonist binding conforms to the Langmuir isotherm. Both the activation step and the deactivation step are regarded as being irreversible, and the complete on/off cycle requires a source of driving energy from the cell. The analysis gives an economical account of the relationship between the intrinsic activity of an agonist (I) and its pharmacological shift ratio (P) (the ratio of concentrations of the agonist needed for half-maximal occupancy of the receptor and half-maximal effect on the adenylate cyclase). The dose-response relationships are "normal," that is, have the shape of the Langmuir isotherm. Both I and P are shown to be simple functions of the coupling factor (F_c), which is defined in terms of the rate constant for the activation of the adenylate cyclase by the agonist·receptor complex (K_c), the rate constant of the basal deactivating system (B) and the planar concentration of receptors (R). These relationships are: $F_c = K_cR/B$; $I = F_c/(F_c + 1)$; $P = F_c + 1$. Two or more agonists acting on different activating receptors interact to reduce their apparent coupling factors. The coupling factor for agonists which deactivate the adenylate cyclase (F'_c) includes a term dependent upon the activating agonist being used. The deactivating intrinsic activity (I') and pharmacological shift ratio (P') are related to F'_c in the same way as those for activating agonist. Slowly and rapidly reversing antagonists are demonstrated to have an effect which is less than their occupancy of the receptor by a factor related to the pharmacological shift ratio of the agonist used. The trajectory with which the activity of the adenylate cyclase approaches its equilibrium value after the addition of an agonist is a complex function, but initially the rate constant of this trajectory exceeds that of the binding of the agonist to its receptor by a factor related to F_c and the final occupancy of the receptor. The model is compatible with many of the general features of the control by multiple receptors of the adenylate cyclase observed in the intact cell. It predicts that interventions that alter B will increase or decrease the sensitivity of the cell to all agonists operating on the adenylate cyclase system, and that activating and deactivating agonists will synergistically accelerate the consumption of driving energy. The model may be applicable to other pharmacological systems in which the receptors are allozymes, i.e., catalysts which are regulated by external stimuli.

INTRODUCTION

The mechanism by which pharmacological receptors modulate the activity of a cell's adenylate cyclase has been studied intensively in a number of tissues (1–4). This work, together with our own studies (5–8) and those of others (9, 10) using human blood platelets, suggests that the following generalities apply to intact cells: (a) Each enzyme unit can be regulated simultaneously by any number of receptors with specificity for a variety of agonists. (b) The action of these receptors is either to activate or to deactivate the adenylate cyclase. (c) The dose response for a given agonist is "normal"; that is, the shape of the curve conforms to the Langmuir isotherm. (d) Agonists (and antagonists) binding to receptors of intact cells with "normal" kinetics, implying that recep-

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tors exist predominantly in one affinity state, and altered affinity forms do not accumulate. (e) The half-maximal effect of an agonist requires less than half-maximal saturation of its receptors (e.g., refs. 8 and 11–13). (f) The number of catalytic units which are affected may be more than the number of receptors occupied by agonists (11). (g) Occupation of one type of receptor by an agonist does not influence the affinity of another receptor type for its corresponding agonist (8). (h) The delay between occupation of a receptor by an agonist and the resultant change in activity of the adenylate cyclase may be very short (i.e., less than a few seconds; e.g., ref. 5).

The lack of equality between the occupancy of a receptor and its effect is clearly not compatible with the concept of signal transmission within a 1:1 stoichiometric complex of receptors and catalytic units, and suggests that the catalytic unit and the receptors are independently mobile in the membrane (14, 15). Furthermore, the agonist cannot induce the formation of a persistent complex between the receptor and the adenylate cyclase, because analysis of the equilibria of such an induced complex shows that it would display a higher affinity for the agonist than do the uncomplexed receptors, which would give rise to distorted binding curves (Model III of ref. 16). The transmission of both activating and deactivating pharmacological signals is known to involve a third component, a guanine nucleotide-binding protein ("*N* protein"). In the absence of guanine nucleotides, *N*-proteins interact with the receptors and increase their affinity for agonists (1); in the intact cell with its normal intracellular concentration of nucleotides it is not clear whether *N*-proteins are complexed with the receptors or with the catalytic unit; they probably do not float freely, nor are they transferred from receptor to catalytic unit, since these arrangements can be shown to result in a distortion of the dose-response curves (17). Thus, if our generalities are accepted, *N*-proteins must be more or less permanently associated with either the catalytic units or with the receptors.

On the basis of a study of the rate of activation of the avian erythrocyte adenylate cyclase in the presence of nonhydrolyzable analogues of GTP and the modulation of this process by *beta*-adrenergic receptors, Levitzki and his colleagues proposed that the enzyme becomes activated by a brief collision with an agonist-occupied receptor (16). In this paper I examine the mathematical consequences of an extension of this model, the allozyme[1] hypothesis.

THE MODEL

It is assumed that the catalytic unit of the adenylate cyclase is activated by a brief collision with an agonist-occupied activating receptor and remains active until it is deactivated either by a brief collision with an agonist-occupied deactivating receptor or with a postulated basal deactivating system. The receptor therefore has a catalytic role, which is regulated by the agonist and which switches the adenylate cyclase between its two stable states. An energy source is necessary to drive the system,

[1] Allozyme is a contraction of allonomous enzyme, i.e., an intracellular catalyst whose action is regulated by an external stimulus.

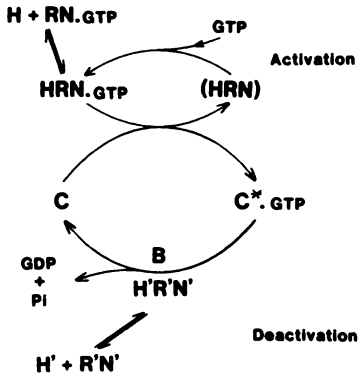

FIG. 1. *One of many schemes for the activation and deactivation of the adenylate cyclase which satisfy the allozyme hypothesis*

The only hormone receptor complexes that accumulate are *HRN*·GTP (activating) and *H'R'N'* (deactivating). The complexes with the adenylate cyclase which switch it between the inactive form and the active form are so short-lived that they do not perturb the binding of agonists (cf. ref. 18).

and activating and deactivating receptors will accelerate this energy consumption synergistically.

There are many plausible schemes which satisfy the postulates of this hypothesis, i.e., that only one species of agonist·receptor complex accumulates, and that this complex catalyzes the switching of the adenylate cyclase. Figure 1 sets out one such scheme derived from that of Cassel *et al.* (18).

The model is compatible with all of the generalities laid out above, and gives rise to a number of predictions concerning the relationship between the intrinsic activity of an agonist and the degree of disparity between the occupancy of a receptor and its effect on the adenylate cyclase.

ASSUMPTIONS

In the analysis we assume that a receptor reversibly binds agonists or antagonists to the same single site, and that the occupation of one receptor has no influence over the properties of any other receptor. The subsequent reactions take place in the lipid bilayer at rates proportional to the planar concentrations of the reactants, and any complexes formed during the course of these reactions are transient and they do not accumulate to a significant extent. Receptors are inactive unless they are agonist-occupied. Activating receptors have a negligible effect on activated adenylate cyclase units. Equally, deactivating receptors have no effect on inactive catalytic units, so that the system is normally irreversible. The energy source and the availability of the *N*-proteins or other components are not rate-limiting.

MATHEMATICAL ANALYSIS

The rate of activation of the adenylate cyclase is the product of the concentration of agonist-occupied activating receptors and the concentration of unactivated adenylate cyclase units:

$$V_{act} = \frac{K_c R}{1 + \frac{K_s}{s}} (C^0 - C^*) \qquad (1)$$

where K_c is a rate constant, R is the planar concentration of activating receptors, C^0 and C^* are, respectively, the planar concentration of adenylate cyclase units and the concentration of those that are activated. The denominator $1 + (K_s/s)$ is from the Langmuir isotherm (19), where K_s and s are the dissociation constant for the agonist and the concentration of free agonist, respectively.

Similarly, the rate of deactivation is the sum of the activity of the deactivating receptor and the basal deactivating system:

$$V_{deac} = \left(\frac{K'_c R'}{1 + \frac{K_i}{i}} + B \right) C^* \qquad (2)$$

where K_i and i are the dissociation constant and concentration of the deactivating agonist.

Activating receptors. Let us first consider the situation in which no deactivating agonist is present, that is $i = 0$. Equation 2 simplifies to:

$$V_{deac} = BC^* \qquad (3)$$

After addition of an activating agonist, the degree of activation of the adenylate cyclase will approach a constant value. This is not a thermodynamic equilibrium, since the reactions are considered to be irreversible, but a steady state in which there is a continuous consumption of energy. In this state the velocity of activation and deactivation are equal, hence the right sides of Eqs. 1 and 3 are equal:

$$\frac{K_c R}{1 + \frac{K_s}{s}} (C^0 - C^*) = BC^*$$

Rearranging:

$$C^* \left(B + \frac{K_c R}{1 + \frac{K_s}{s}} \right) = C^0 \frac{K_c R}{1 + \frac{Ks}{s}}$$

and:

$$\frac{C^0}{C^*} = \frac{B\left(1 + \frac{K_s}{s}\right)}{K_c R} + 1$$

At this point we define a term, the coupling factor,

$$F_c = \frac{K_c R}{B} \qquad (4)$$

Substituting:

$$\frac{C^0}{C^*} = \frac{1 + \frac{K_s}{s}}{F_c} + 1 = \left(1 + \frac{1}{F_c}\right)\left(1 + \frac{K_s}{s(F_c + 1)}\right)$$

Inverting:

$$\frac{C^*}{C^0} = \left(\frac{F_c}{F_c + 1}\right)\left(\frac{1}{1 + \frac{K_s}{s(F_c + 1)}}\right) \qquad (5)$$

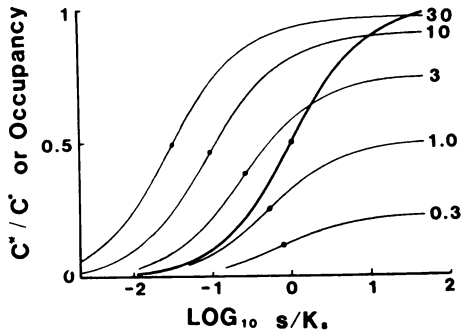

FIG. 2. *Log dose-response plots of the activation of the adenylate cyclase at different values of F_c.*

The *heavy line* is the occupancy of the receptor. Half-maximal activation is indicated by the *point*. The value of F_c is given to the right of each curve. If the effect were to be plotted as a fraction of the effect achieved by a saturating concentration of agonist, a series of parallel curves would result, displaced to the *left* of the occupancy curve.

C^*/C^0 is the fractional activation of the adenylate cyclase. A plot of this function is shown for various values of F_c in Fig. 2. Note that when s is high, the activation of the cyclase reaches a limiting value, given by the contents of the left parentheses, which we define as the intrinsic activity of the agonist:

$$I = \frac{F_c}{F_c + 1} \qquad (6)$$

The right parentheses of Eq. 5 contain a form of the Langmuir isotherm and therefore describe the usual saturation curve, with half-maximal activation when $s(F_c + 1) = K_s$. Thus the concentration for half-maximal effect is given by $K_s/(F_c + 1)$.

When the degree of occupation of the receptor is plotted versus the logarithm of the agonist concentration, a sigmoid curve results; if the degree of activation of the adenylate cyclase is expressed as a ratio of the maximal activation possible with that agonist (i.e., the right parentheses of Eq. 5) and plotted on the same axis, a parallel sigmoid is obtained, shifted to a lower concentration by the ratio $F_c + 1$. We define this ratio as the pharmacological shift ratio.

$$P = F_c + 1 \qquad (7)$$

Thus for any agonist we can write a relationship between its intrinsic activity and its pharmacological shift ratio (from Eqs. 6 and 7):

$$I = \frac{F_c}{F_c + 1} = 1 - \frac{1}{P} \qquad (8)$$

and

$$P = \frac{1}{1 - I} \qquad (9)$$

Relationship between occupancy and effect. It is also of interest to derive the relationship between the occupancy of a receptor by the agonist and its effect on the cyclase, since both can be observed experimentally. The occupancy is given by the isotherm:

$$O = \frac{1}{1 + \frac{K_s}{s}} \qquad (10)$$

from which:

$$\frac{K_s}{s} = \frac{1 - O}{O}$$

Substituting this in Eq. 5 gives the fractional activation of the cyclase:

$$\frac{C^*}{C^0} = \left(\frac{F_c}{F_c + 1}\right)\left(\frac{1}{1 + \frac{1 - O}{O\,(F_c + 1)}}\right) = \frac{F_c O}{F_c O + 1} \qquad (11)$$

The effect of an agonist at a certain degree of saturation of the receptor can also be expressed as a fraction of the maximal effect possible with that agonist, that is, a fraction (E) of the intrinsic activity. This can be obtained by substituting $(1 - O)/O$ for K_s/s in the right parentheses of Eq. 5:

$$E = \frac{1}{1 + \frac{1 - O}{O\,(F_c + 1)}} = \frac{F_c O + O}{F_c O + 1} \qquad (12)$$

This equation can be rewritten in a suitable form to derive F_c from experimental data. Thus:

$$EF_cO + E = F_cO + O$$

Hence:

$$F_c = (E - O)/(O - OE) \qquad (13)$$

Note that both E and O are normalized, that is, O is the fraction of receptors occupied and E is the activity of the cyclase expressed as a fraction of the maximal activity obtained with the saturating concentration of the agonist. If the latter is in doubt, as it may be if the agonist has more than one action or when intact cells are used, then a derivation of Eq. 11 may be more useful:

$$C^*F_c + C^*/O = C^0F_c \qquad (14)$$

or:

$$C^*/O = C^0F_c - C^*F_c \qquad (15)$$

Thus a plot of C^*/O against C^* yields a straight line with slope $-F_c$ and with intercepts of F_cC^0 and C^0; C^0 is thus determined experimentally. Two other plots can be used with these data:

$$1/C^* = 1/C^0 + 1/OF_cC^0 \qquad (16)$$

plotting $1/C^*$ versus $1/O$ and:

$$O/C^* = O/C^0 + 1/F_cC^0 \qquad (17)$$

plotting O/C^* versus O.

The plot based on Eq. 17 would appear to be the most manageable, especially when F_c is not too far removed from unity. It is illustrated in Fig. 3. When F_c is high, the most useful data are obtained at low occupancy, and the plot may be truncated. If accurate measurement of occupancy in this range cannot be obtained, then normalized occupancy data and effect data can be plotted on the same log dose-response plot. F_c can then be determined using Eq. 7.

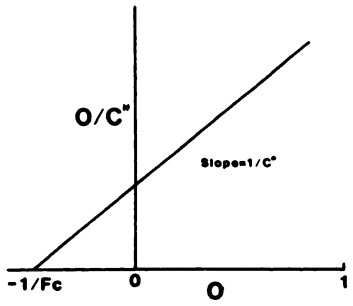

FIG. 3. *Plot to derive the value of the coupling factor (F_c) from measurements of the occupancy (O) of a receptor by an agonist and the rate of cyclic AMP generation (C^*) according to Eq. 17*

Maneuvers which alter the value of F_c result in a series of parallel lines. Maneuvers which change the maximal rate of cyclic AMP production, such as destruction of catalytic units, will give rise to a series of lines with a common intercept on the horizontal axis. Nonlinear plots will result if the agonist acts on more than one receptor type.

Interaction between activating receptors. The effect of exposing the cell to more than one activating agonist, each acting on its own receptor, can be computed by summing the contribution of each receptor·agonist pair to the rate of activation of the adenylate cyclase:

$$V_{act} = \sum \frac{K_cR}{1 + \frac{K_s}{s}} \cdot (C^0 - C^*)$$

At steady state,

$$V_{act} = V_{deac} = BC^*$$

Therefore:

$$\sum \frac{K_cR}{1 + \frac{K_s}{s}} \cdot (C^0 - C^*) = BC^*$$

or:

$$\sum \frac{K_cR}{B\left(1 + \frac{K_s}{s}\right)} = \frac{C^*}{C^0 - C^*}$$

But for each receptor·agonist pair:

$$\frac{K_cR}{B\left(1 + \frac{K_s}{s}\right)} = F_cO \text{ (from Eqs. 4 and 10)}$$

from which we can show that the degree of activation of the adenylate cyclase:

$$\frac{C^*}{C^0} = \frac{\sum F_cO}{\sum F_cO + 1} \qquad (18)$$

We can also compute the effect of the occupation of one receptor (x) on the normalized responsiveness of the cell to a second activating agonist acting on another receptor (y). If we use X and Y to denote the product F_cO for the x and y receptors, respectively, then the activation of the adenylate cyclase in the presence of the

respective agonists can be written as follows:

$$C^*_{(x)} = C^0\frac{X}{X+1} \text{ (cf. Eq. 11)}$$

$$C^*_{(y)} = C^0\frac{Y}{Y+1}$$

$$C^*_{(x,y)} = C^0\frac{X+Y}{X+Y+1} \text{ (from Eq. 18)}$$

$$C^*_{(x,y)} - C^*_{(x)} = C^0\left(\frac{X+Y}{X+Y+1} - \frac{X}{X+1}\right)$$

$$C^0 - C^*_{(x)} = C^0\left(1 - \frac{X}{X+1}\right) = C^0\frac{1}{X+1}$$

Thus the normalized fractional effect of the occupation of receptor y is given by:

$$\frac{C^*_{(x,y)} - C^*_{(x)}}{C^0 - C^*_{(x)}} = \left(\frac{X+Y}{X+Y+1} - \frac{X}{X+1}\right)(X+1) \quad (19)$$

$$= \frac{Y}{X+Y+1}$$

which may be written in the form:

$$\frac{Y\left(\frac{1}{X+1}\right)}{Y\left(\frac{1}{X+1}\right)+1}$$

and compared with Eq. 11.

It is apparent that the observed value of F_cO for y is decreased by a factor equal to the (initial) value of $F_cO + 1$ for x. Since the occupancy of receptor y is unaffected by occupation of receptor x, then this change is due to an apparent decrease in the value of F_c for y. Note that this effect is symmetrical—the observed value of F_c for x is also reduced by the presence of agonist y.

Deactivating receptors. We can derive expressions for the effect of receptors that deactivate the adenylate cyclase in the same way as for activating receptors. For simplicity we rewrite Eq. 1 as follows:

$$V_{act} = (C^0 - C^*)Q_s$$

where

$$Q_s = \frac{K_cR}{1+\frac{K_s}{s}}$$

In the absence of a deactivating agonist and at steady state:

$$V_{deac} = C^*B = V_{act}$$

Therefore:

$$Q_sC^0 = C^*(Q_s + B) \quad (20)$$

In the presence of an inhibitory agonist, the rate of deactivation is the sum of the basal rate and that due to the occupied receptor:

$$V_{deac} = \left(\frac{K'_cR'}{1+\frac{K_i}{i}} + B\right)C'$$

where C' is the concentration of activated adenylate cyclase in the presence of the deactivating agonist at concentration i. The rate of activation is given by:

$$V_{act} = (C^0 - C')Q_s$$

At steady state:

$$V_{act} = V_{deac}$$

Therefore:

$$C'\left(Q_s + \frac{K'_cR'}{1+\frac{K_i}{i}} + B\right) = Q_sC^0$$

but:

$$Q_sC^0 = C^*(Q_s + B) \text{(from Eq. 20)}$$

Therefore:

$$\frac{C^*}{C'} = 1 + \frac{K'_cR'}{(Q_s+B)\left(1+\frac{K_i}{i}\right)} \quad (21)$$

If we define a deactivating coupling factor,

$$F'_c = \frac{K'_cR'}{Q_s+B}$$

this equation becomes:

$$\frac{C^*}{C'} = 1 + \frac{F'_c}{1+\frac{K_i}{i}}$$

from which we can show that the fractional inhibition of the adenylate cyclase caused by an agonist for the deactivating receptor:

$$\frac{C^* - C'}{C^*} = \left(\frac{F'_c}{F'_c+1}\right)\left(\frac{1}{1+\frac{K_i}{i(F'_c+1)}}\right) \quad (22)$$

which is once again a formulation of the Langmuir isotherm. The maximal possible degree of inhibition, or the intrinsic activity of the agonist, is given by the left parentheses:

$$I' = \frac{F'_c}{F'_c+1} \quad (23)$$

and the pharmacological shift ratio is:

$$P' = F'_c + 1 \quad (24)$$

Note that these relationships are identical with those for agonists which activate the cyclase (Eqs. 6 and 7).

Effect of irreversible or slowly dissociating antagonists. If the cell is preincubated with an agent that blocks the action of the agonist on the receptor, either by occupying the binding site or otherwise, the effect is to reduce the concentration of available receptors. We con-

sider the case in which an antagonist at concentration a slowly equilibrates with the receptor with the dissociation constant K_a. When equilibrium is achieved, the functioning of the adenylate cyclase is measured during exposure to the agonist for a brief period in which it is assumed that the dissociation of the agonist is negligible.

At equilibration with the antagonist, the proportion of receptors still available to the agonist is given by:

$$R_a = R^0 - \frac{R^0}{1 + \frac{K_a}{a}}$$

where R^0 is the initial concentration of receptors. Therefore:

$$\frac{R_a}{R^0} = \frac{1}{\frac{a}{K_a} + 1} \tag{25}$$

The value of the coupling factor in the presence of the antagonist, F_{ca}, changes by the same ratio, since F_c is directly proportional to the number of available receptors:

$$F_{ca} = \frac{F_c}{\frac{a}{K_a} + 1} \tag{26}$$

The intrinsic activity after equilibration with the antagonist is given by:

$$I_a = \frac{F_{ca}}{F_{ca} + 1} \text{ (from Eq. 6)}$$

Substituting the value for F_{ca} from Eq. 26 and simplifying:

$$I_a = \frac{F_c}{F_c + \frac{a}{K_a} + 1} \tag{27}$$

Thus the degree of inhibition of the intrinsic activity of the agonist due to the antagonist is given by:

$$\frac{I - I_a}{I} = \frac{1}{1 + \frac{K_a(F_c + 1)}{a}} \text{ (From Eqs. 27 and 6)} \tag{28}$$

which is a form of the Langmuir isotherm. Note that in this case the effect of the noncompetitive antagonist is *less* than its occupancy of the receptor, but by the same pharmacological shift ratio, $F_c + 1$, as the effect of the agonist *exceeds* the occupancy.

Similarly, we can compute that the fractional reduction in the pharmacological shift ratio:

$$\frac{P - P_a}{P} = 1 - \frac{F_{ca} + 1}{F_c + 1} = \frac{F_c - F_{ca}}{F_c + 1} \text{ (from Eq. 7)}$$

When F_c is high, $F_c + 1 \doteq F_c$ and therefore:

$$\frac{P - P_a}{P} \doteq \frac{F_c - F_{ca}}{F_c}$$

Substituting from Eq. 26 and simplifying:

$$\frac{P - P_a}{P} \doteq \frac{1}{1 + \frac{K_a}{a}} \tag{29}$$

which is the equation for the occupancy of the receptor by antagonist. Thus for agonists with a high pharmacological shift ratio, this ratio is reduced approximately proportionally to the occupancy of the receptor by irreversible antagonist.

We can display these results graphically in a fashion analogous to that used by Dixon (20). The occupancy of the receptor by agonist in the presence of an irreversible antagonist is given by:

$$O_a = \left(\frac{1}{1 + \frac{K_s}{s}} \quad \frac{1}{\frac{a}{K_a} + 1}\right)$$

(multiplication of Eqs. 10 and 25)

Substituting this in Eq. 11 and inverting gives:

$$\frac{C^0}{C_a} = \frac{F_c + \left(1 + \frac{K_s}{s}\right)\left(\frac{a}{K_a} + 1\right)}{F_c} \tag{30}$$

Thus a plot of $1/C_a$ versus a gives a straight line for any value of s. Note that when $a = -K_a$, $C_a = C^0$ so that the lines intersect at each other at $a = -K_a$ and $1/C_a = 1/C^0$. The slopes of the lines are

$$\frac{1}{C^0 K_a F_c}\left(1 + \frac{K_s}{s}\right)$$

with intercepts on the vertical axis (i.e., $a = 0$) of

$$\frac{1}{C^0} + \frac{1}{C^0 F_c} + \frac{K_s}{C^0 s F_c}$$

(Fig. 4).

Effect of competitive antagonists. When a receptor is allowed to equilibrate with an agonist and a competitive antagonist, then the occupancy by the agonist is given

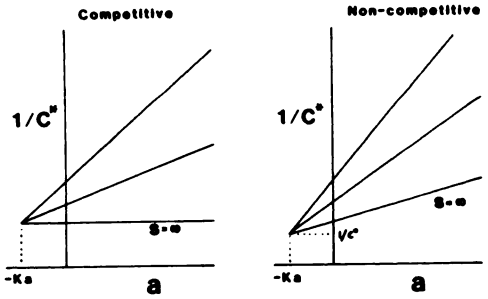

FIG. 4. *Dixon plots of competitive and noncompetitive antagonism*
The lowest of the three lines is the result at a saturating concentration of agonist. Note that, when a noncompetitive antagonist is used, both K_a and C^0 can be derived from this plot. The equations of the intercepts and slopes are given in the text.

by:

$$O_a = \frac{1}{1 + \frac{K_s}{s}\left(\frac{a}{K_a} + 1\right)} \text{ (cf. Eq. 18 of ref. 21)}$$

Increasing the value of s can always compensate for increases in a, and at high concentrations of s, O_a approaches unity. Thus the intrinsic activity of an agonist is not changed by the presence of a competitive antagonist. We can display the activity of the cyclase by graphical methods analogous to those used above. Substituting the value of O_a in Eq. 11 and inverting gives:

$$\frac{C^0}{C_a} = \frac{F_c + 1 + \frac{K_s}{s}\left(\frac{a}{K_a} + 1\right)}{F_c} \tag{31}$$

Thus a plot of the reciprocal of the activity versus the antagonist concentration yields straight lines for any value of s. Note that in this case when $a = -K_a$, C^0/C_a takes on the value $(F_c + 1)/F_c$, so that as before the lines intersect at $a = -K_a$. The slopes of the lines are $K_s/(sF_cK_a)$ and the intercepts on the vertical axis are given by $1 + 1/F_c + K_s/sF_c$. When s is large, the slope approaches zero (Fig. 4).

The degree of inhibition of the cyclase by a competitive antagonist at a fixed concentration of an agonist can be computed as follows:

In the absence of the antagonist:

$$\frac{C^*}{C^0} = \frac{F_cO}{F_cO + 1} \text{ (from Eq. 11)}$$

and in the presence of antagonist:

$$\frac{C_a}{C^0} = \frac{F_cO_a}{F_cO_a + 1}$$

Therefore the degree of inhibition:

$$\frac{C^* - C_a}{C^*} = \frac{\frac{1}{O_a} - \frac{1}{O}}{F_c + \frac{1}{O_a}} \tag{32}$$

but

$$\frac{1}{O} = 1 + \frac{K_s}{s}$$

and

$$\frac{1}{O_a} = 1 + \frac{K_s}{s}\left(\frac{a}{K_a} + 1\right)$$

Substituting and simplifying:

$$\frac{C^* - C_a}{C^*} = \frac{1}{1 + \frac{K_a}{a}\left(\frac{s(F_c + 1)}{K_s} + 1\right)} \tag{33}$$

This is a form of the Langmuir isotherm, and half-maximal inhibition occurs when $a = K_a\left(\frac{s(F_c + 1)}{K_s} + 1\right)$. Note that this value is *higher* than that for half-maximal occupancy.

Approach to equilibrium. So far we have considered only the effects of receptors on the activity of the adenylate cyclase at a time when receptors have equilibrated with their ligands and the rate of activation of the adenylate cyclase has equaled the rate of deactivation. Some preparations of tissues bearing on adenylate cyclase respond slowly after addition of an agonist, even when sufficient agonist is added to saturate the receptor rapidly. However, in other tissues the response of the adenylate cyclase is fast compared with the time it takes for the agonist to bind. In this case we can compute the trajectory of the adenylate cyclase to the steady state.

The occupancy of the receptor at time t is given by $O_t = O(1 - e^{-kt})$ where k is given by $sk_1 + k_{-1}$ (Eq. 10 of ref. 21). When t is small, then $O_t \simeq O_{kt}$. If we assume that the adenylate cyclase response is rapid compared with the binding of an agonist, then the fractional activation at time t is given by:

$$\frac{C_t}{C^*} = \frac{F_c + \frac{1}{O}}{F_c + \frac{1}{O_t}} \text{ (by analogy with Eq. 12)} \tag{34}$$

When t is small, we can use the approximation used above for O_t, and also assume that $1/O_t$ is large compared with F_c.

Initially, therefore

$$\frac{C_t}{C^*} \simeq \frac{F_c + \frac{1}{O}}{\frac{1}{O_{kt}}} = (F_cO + 1)kt \tag{35}$$

whereas

$$\frac{O_t}{O} \simeq kt$$

Thus the model predicts that the activity of the adenylate cyclase can approach its equilibrium value faster than does the binding of the agonist to the receptor. When s is high, the ratio of these initial rates of activation and binding will approach a value of $F_c + 1$; when s is small the two trajectories will be similar. This effect is illustrated in Fig. 5, in which the values of s and F_c are chosen to give values of final occupancy and effect equal to 50%.

Similar discrepancies occur between the rate of binding of antagonists to the receptor and the development of their ability to inhibit the action of an agonist. The onset of antagonism is slower than the rate of binding, and the offset of competitive antagonism (revealed by the addition of an excess of agonist) is faster than the dissociation of the antagonist from the receptor. These trajectories are complex functions and will not give straight lines on semilogarithmic progress plots.

DISCUSSION

The approach I have adopted in the mathematical analysis leads to somewhat simpler equations than does

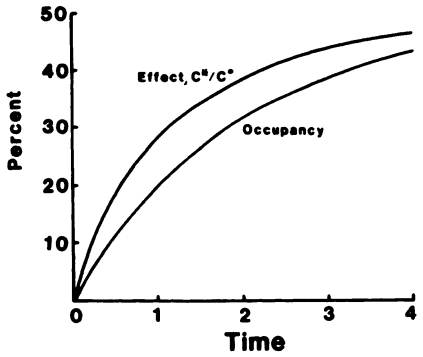

FIG. 5. *Time course of approach to steady-state levels*

The agonist is added at time zero, at a concentration equal to its dissociation constant. The occupancy therefore approaches 50% exponentially; $O = 0.5\ (1 - e^{-kt})$, where k is arbitrarily set to unity. The effect is computed from the occupancy using Eq. 11. F_c is set equal to 2, so that the effect also tends to 50% activation of the adenylate cyclase. In this example, the initial rate of adenylate cyclase activation is twice the rate of receptor occupancy. The trajectory of the effect is not exponential.

the more usual analysis of equilibria from kinetic constants. This simplification arises from our assumption that the catalytic events leading to activation or deactivation can be considered to be irreversible; i.e., the rates of the reverse reactions are insignificant compared with the rates of the forward reactions. This approach is intuitively justified for the cycle as a whole by considering the improbability of reversing the cycle in Fig. 1 and generating GTP from GDP.

We have also assumed that the interactions between the agonist·receptor complexes and the catalytic unit are so brief that they do not significantly perturb the binding of the agonist to the receptor. The binding curves are therefore normal; that is, they conform to the Langmuir isotherm and give straight Scatchard plots.

The mathematical analysis shows that in all cases the concentration dependence of the effect of an agonist on adenylate cyclase is also normal, and when the observed values are scaled between the maximal and minimal effects they will give straight lines on double reciprocal plots, etc. However, the concentration of agonist required for half-maximal effect is always less than the concentration required for half-maximal binding. To put it another way, the fractional effect of an agonist always exceeds its fractional occupancy of the receptor. I suggest the term pharmacological shift ratio (P) to denote the ratio between the concentrations of agonist giving half-maximal occupany and half-maximal effect.

Saturating concentrations of agonists do not activate (or deactive) all of the available adenylate cyclase catalytic units, and the term intrinsic activity (I) is defined as the proportion of catalytic units that are switched on (or off) when the receptors are saturated with a particular agonist.

The effects of a receptor·agonist combination on the adenylate cyclase is described in terms of a coupling factor (F_c), which can take any positive value and is numerically equal to the empirical term "efficacy" used by Stephenson (22). The coupling factor is directly related to the planar concentration of receptors and to a rate constant (K_c) which would appear to be a function of membrane fluidity and the properties of the agonist (for antagonists, its value is zero). The coupling factors of all of the receptors on the cell are decreased by increasing the rate constant of the basal deactivating system (B). If this system is disabled, the adenylate cyclase will become exquisitely sensitive to both activating and deactivating receptors. B corresponds to the k_{off} determined experimentally by Cassel *et al.* (18).

The coupling factor, intrinsic activity, and pharmacological shift ratio are related to each other mathematically. When the coupling factor is zero, the intrinsic activity is zero and the pharmacological shift ratio is 1. As the coupling factor increases, so does the pharmacological shift ratio, and the intrinsic activity tends toward unity. The value of F_c can be determined experimentally by simultaneously observing the occupancy of a receptor by an agonist and the effect of the agonist on the adenylate cyclase, and three graphical methods are given for processing the data. One of these (Eq. 15) was used by Homburger *et al.* (Fig. 8b of ref. 13) to determine a function equivalent to the coupling factor.

The significance of the coupling factor for deactivating receptors is the same as that for activating receptors, but since the deactivating receptors oppose the action of the activating receptors, the value of the deactivating coupling factor decreases as the degree of stimulation of the adenylate cyclase by the activating receptor increases. A similar effect occurs when a mixture of agonists acting on more than one type of activating receptors is added; the resultant effect is rather less than expected by simple addition, and occupation of one activating receptor will reduce the apparent coupling factor of a second activating receptor.

The effect of competitive antagonists is relatively straightforward, since they cause a parallel shift in the dose response of the agonist to the right, but their pharmacological effect is less than their occupancy of the receptor by a function of the pharmacological shift ratio of the agonist being used. Their true affinity for the receptor can be determined by Dixon's graphical method (20). Irreversible (and slowly reversing) antagonists produce effects similar to those of a mixed inhibitor in enzyme kinetics, reducing both the apparent intrinsic activity and pharmacological shift ratio of the agonist being used. Their affinity constants can also be obtained by Dixon's plot.

The trajectory with which the activity of the adenylate cyclase approaches its final value after the addition of an agonist is complex. If the agonist equilibrates slowly with its receptor, the rate constant of the activation trajectory is actually faster than the rate constant for binding, especially at high agonist concentrations. The trajectory falls somewhere between exponential and hyperbolic, and is not analyzed in detail here. Nevertheless, much of the trajectory data accounted for by the matrix coupling model of Bergman and Hechter (23) would appear to be equally explicable by the hypothesis considered here.

The allozyme hypothesis is compatible with all of the generalities set out in the Introduction, and the analysis of the hypothesis generates a number of predictions about the behavior of the adenylate cyclase. In some

tissue preparations it may be possible to obtain data accurate enough to test these predictions, but in many cases the data may be confounded by a non-uniform distribution of receptor density (which would flatten dose-response curves) and the existence of more than one receptor for a given agonist which may have opposing actions. Whether or not the hypothesis is applicable to the adenylate cyclase depends upon the detailed elucidation of the mechanism of action of the receptors and the demonstration that they act catalytically to activate or deactivate the adenylase cyclase.

Although we have derived the equations in terms of the adenylate cyclase, the hypothesis in general can be applied to any pharmacological system in which only one form of the agonist·receptor complex accumulates, and when this complex acts catalytically to alter the state of its target enzyme or membrane pore.

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APPENDIX

Table of Symbols

Concentrations.

s, i, a: Volumetric concentration of activating agonist, deactivating agonist, and antagonist

K_s, K_i, K_a: The corresponding dissociation constants

R, R': Planar concentration of activating and deactivating receptors (e.g., number per square micrometer or per cell)

C^0, C^*, C', C_a: Planar concentration of adenylate cyclase catalytic units: total, activated in the presence of activating agonist, activated in the presence of deactivating agonist, and activated in the presence of antagonist; can be expressed in terms of activity

Unitless terms. The prime superscript (′) indicates deactivating receptor, a subscript indicates result in the presence of antagonist.

F_c: Coupling factor = RK_c/B for activating agonists; see section on deactivating receptors for definition of F'_c

O: Occupancy: fractional occupancy of receptor by agonist

I: Intrinsic activity: fraction of catalytic units influenced when receptor is saturated by agonist = $F_c/(F_c + 1)$

E: Fractional effect: the effect of a concentration of agonist expressed as a fraction of its intrinsic activity

P: Pharmacological shift ratio: ratio between concentration of agonist for half-maximal occupancy and half-maximal effect = $F_c + 1$

Rates.

V_{act}, V_{deac}: Rate of activation and deactivation of adenylate cyclase catalytic units (planar concentration, time^{-1})

K_c, K'_c: Rate constant of receptor action on the adenylate cyclase (time^{-1}, receptor concentration^{-1})

B: Rate constant of basal deactivating system (time^{-1})

Q_s: Rate constant of activating receptor, used in computation of effect of deactivating receptor

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