0026-895X/93/030397-15$03.00/0

MOLECULAR PHARMACOLOGY, 43:397–411

Analysis of Receptor-Mediated Activation of GTP-Binding Protein/Adenylate Cyclase using the Encounter Coupling Model

DOUGLAS STICKLE[1] and ROGER BARBER

Laboratories of Cyclic Nucleotide Research, Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, Texas 77225

Received July 13, 1992; Accepted November 25, 1992

SUMMARY

The rate of adenylate cyclase activation via agonist-bound receptors in intact cells can be partly dependent on the rate of turnover of occupancy by agonist with respect to individual receptors. For instance, low occupancy of the full complement of receptors by epinephrine in intact S49 cells has been shown to promote a rate of activation that is substantially greater than that for high occupancy of a small number of receptors for which the concentration of epinephrine-bound receptors is the same. According to the encounter coupling model, a partial dependence of the relationship between receptor occupancy and adenylate cyclase activity on the agonist binding frequency can in principle be explained by episodic interactions of finite duration (encounters) between individual pairs of receptor and GTP-binding protein. The mean lifetime of the agonist-receptor complex and the frequency of binding relative to the mean duration of such encounters dictate whether there is variation of the state of the receptor during an encounter and the extent to which the overall rate of GTP-binding protein activation can be dependent on binding frequency. We present here a quantitative analysis of agonist concentration versus cyclase response curves in terms of the encounter coupling model that explicitly includes agonist binding frequency, the encounter frequency, and the encounter duration as parameters. The essential result is that the model is quantitatively consistent with concentration versus response curves for receptor-mediated activation of adenylate cyclase in S49 cells. It is also shown that the model is consistent with data on the differential effects of antagonists to inhibit agonist-stimulated cyclase activation in a manner that is dependent on the antagonist binding frequency.

β-Adrenergic agonists stimulate the production of the second messenger cAMP by adenylate cyclase, via a pathway that involves the activation of G by agonist-bound receptors. We have previously reported evidence for the influence of agonist binding frequency on the relationship between β-adrenergic receptor occupancy and the activation of adenylate cyclase in intact S49 cells, for certain agonists (1, 2). In response to epinephrine, for instance, relatively low occupancy of the full number of receptors promotes a rate of adenylate cyclase activation that is considerably greater than that observed for the circumstance in which an equivalent concentration of epinephrine-bound receptors is produced by full occupancy of a small fraction of the receptors (1). It is apparent, then, that within the "normal" epinephrine concentration versus response curve (in which an unmodified basal number of receptors are available to interact with a fixed concentration of epinephrine) there is a component of the response that is dependent in some way on the time-dependent distribution of receptor occupancy among the receptor population.

This work was supported by National Institutes of Health Grant NS-21338.
[1] Present address: Department of Biological Chemistry, Pennsylvania State University College of Medicine, Hershey, PA 17033.

The evidence for the role of agonist binding frequency in this system has been interpreted in terms of an "encounter" coupling model for β-adrenergic receptor/G interaction (3, 4), which is a modified form of the collision coupling model of Tolkovsky and Levitzki (5, 6). The essential aspects of collision coupling are that the receptors are mobile catalysts for G activation with access to numerous G per unit time and that G inactivation is independent of receptor activity (i.e., that the signal-transducing activity of G, once activated, is not dependent on the continued presence or activity of a receptor). The essential aspects of a normal agonist concentration versus response curve can be explained by collision coupling alone, but collision coupling in an unmodified form cannot explain the influence of agonist binding frequency on G activation. The encounter coupling model adds to the basic tenets of the collision coupling model the provision that the interaction between individual receptor and G pairs has some finite lifetime that is on the order of the lifetime for the receptor-agonist complex for some agonists, to explain the influence of agonist binding frequency.

From a qualitative standpoint it is fairly simple to understand how binding frequency could influence the extent of G activa-

ABBREVIATION: G, GTP-binding protein.

tion per extent of receptor occupancy when the interactions of receptors and G have lifetimes that are on the order of the agonist-receptor complex lifetime. The basic observation is that a greater number of activations of G can occur, per unit time and per overall extent of receptor occupancy, when the occupancy is rapidly shared among the entire receptor population (via high agonist binding frequency) than when it is not. If a specific agonist-receptor complex is relatively efficient at G activation during such an "encounter" of some finite duration, then the high binding frequency case is potentially one in which a lesser fraction of the interaction time between specific pairs of agonist-bound receptors and G is "wasted" in the presence of G that has already become activated, compared with a circumstance in which occupancy is not so rapidly distributed. This principle has been demonstrated in example calculations made for extreme cases of the relationship between the agonist-receptor lifetime and the encounter duration (4).

The purpose of this paper is to examine the predictions of the encounter coupling model in more rigorous and quantitative detail using values for the binding rate constants and estimations of the encounter duration. First, we review in detail the relationship between receptor occupancy and G/cyclase activation in intact cells and the explanation based on the collision coupling model. Second, we outline the need and the rationale for making modifications to the collision coupling model. Next, the modifications themselves are described and developed into the encounter coupling model. Using the encounter coupling model we pursue the following aspects. First, we demonstrate that the encounter coupling model can explain normal agonist concentration versus response curves, using epinephrine as a basis case, and we examine the range of conditions and values of parameters for which the encounter coupling model "works" for the epinephrine concentration versus response curve. Second, it is shown that according to this model the diminution of agonist binding frequency leads to a significant rightward shift of the concentration versus response curve, a phenomenon that has been observed experimentally. Third, we use the encounter coupling model to examine the predicted effects of antagonists on the relationship between receptor occupancy and G activation. The predicted effects are consistent with the results of previously reported experiments in which differential effects of antagonists on agonist-stimulated activity were attributed to antagonist binding frequency.

Theory

Relationship between Receptor Occupancy and Adenylate Cyclase Activity as a Function of Agonist Concentration

It is useful to begin with a discussion of the relationship between receptor binding and cyclase activity in intact cells and to detail the explanation for this relationship that is provided by the collision coupling model. By receptor binding we mean explicitly the fractional occupancy of receptors obtained at steady state in the presence of some fixed concentration of agonist. In the context of the principles involved here, it is useful to think of occupancy not simply as the fraction of receptors bound but, rather, as the average fraction of the receptors that are bound at any one instant in time or the probability that any one receptor is bound at any given instant. By cyclase activity we mean the maximum rate of cAMP generation that is obtained in response to a given concentration of agonist, and fractional activation is the activity expressed as a fraction of the maximum activity that can be obtained with a saturating concentration of agonist. The rate of cAMP generation is derived from measurements of cAMP accumulation in 1 min. Correspondingly, the fractional occupancy of receptors is derived from measurements of binding made on the same time scale (1).

Both the receptor occupancy and the cyclase response can be characterized by simple function of the agonist concentration. The fractional occupancy, θ, of receptors in the presence of a fixed agonist concentration, [A], is given by hyperbolic function of the agonist concentration characterized by the dissociation constant, K_d:

$$\theta = \frac{[A]}{[A] + K_d} \quad (1)$$

The fractional cyclase response curve is also a hyperbolic function of the agonist concentration, characterized by the EC_{50}. The fractional activation f of the enzyme in the presence of a fixed agonist concentration, [A], is given by

$$f = \frac{[A]}{[A] + EC_{50}} \quad (2)$$

In intact cells, the fractional activation of cyclase f at some concentration of agonist is invariably greater than the fractional receptor occupancy θ, i.e., there is not a one-to-one correspondence of receptor occupancy to cyclase activation, and the activity of some fraction of the receptors is amplified into a greater net fractional activation of cyclase. For some agonists, receptors need to be occupied only a small fraction of the time in order to activate near-maximal response by cyclase. For instance, half-maximal adenylate cyclase activity in epinephrine-stimulated intact S49 murine lymphoma cells occurs at a concentration of epinephrine (EC_{50} = 10 nM) that is 200-fold less than the concentration required for 50% receptor occupancy (K_d = 2 μM). The separation between the two curves is usefully characterized by the ratio K_d/EC_{50} (the pharmacological shift ratio, R) (7). Different agonists exhibit different values for R, but the ratio R is invariably greater than 1 ($EC_{50} < K_d$).

Explanation of the Relationship between Receptor Occupancy and Cyclase Response in Terms of the Collision Coupling Method

The collision coupling model developed by Tolkovsky and Levitzki (5, 6) can explain the separation characterizing the relationship between the receptor occupancy and cyclase response curves (Fig. 1). In the collision coupling model the receptors are mobile catalysts for G activation, with access to numerous G per unit time, and G inactivation (which is presumed to correspond to the GTPase activity of the activated G) is independent of receptor activity. Because the inactivation is relatively slow, the activity of G becomes amplified relative to the occupancy of receptors; that is, because it takes a relatively long time to become inactivated, G can be active most of the time even if activation occurs only infrequently.

A specific assumption of the collision coupling model is that the rate of G activation is proportional to the concentration of agonist-bound receptors (5–7). Assuming that the inactivation rate can be characterized by a single rate constant, the fractional G activity at steady state is a balance between the rates of activation and inactivation. The rate constant for inactivation, k_i, is independent of the receptor activity, whereas the activation rate constant, k_a, is dependent on the receptor occupancy. The fractional activity of cyclase, which activity is assumed to be kinetically coupled to G (8), is then given by

$$f = \frac{k_a}{k_a + k_i} \quad (3)$$

In detail, in this model the rate constant for cyclase activation, k_a, is the product of three factors, 1) the collision rate between receptors and G, 2) the probability that a given collision occurs with an occupied receptor, and 3) the probability that a collision between inactive G and agonist-bound receptor will result in activation of G (an efficiency factor). The collision rate (ω) is an agonist-independent factor that is some measure of the concentrations of the two species in the membrane

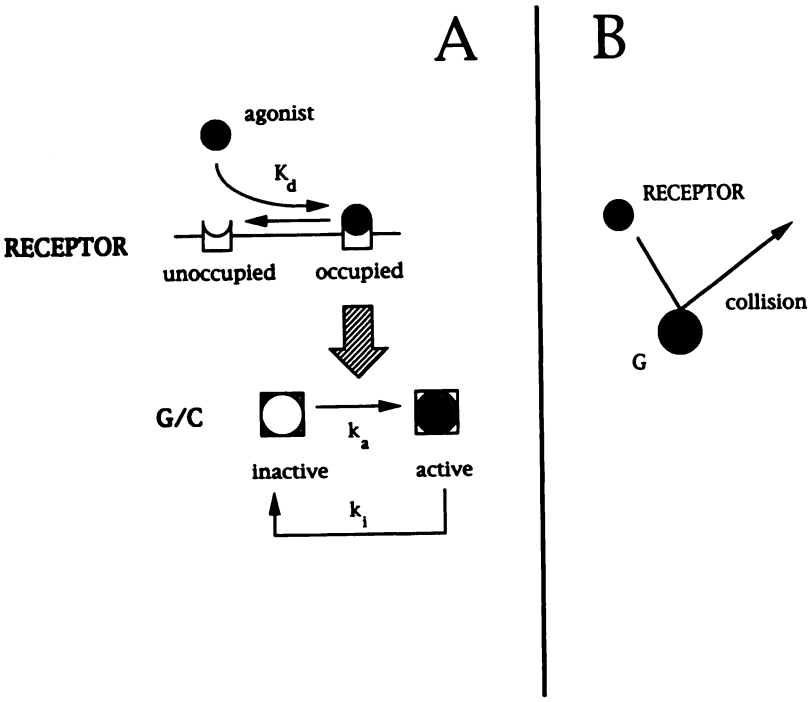

Fig. 1. Collision coupling model. A, Activation and inactivation of G/cyclase (G/C). The rate of activation of G/C is proportional to the concentration of receptors occupied by agonist, with rate constant k_a; the rate of inactivation of G/C is independent of receptor activity, with rate constant k_i. B, Collision. A "collision" between receptor and G is assumed to be instantaneous, such that the interaction between any one receptor and any one G occurs with the receptor strictly either occupied by agonist or unoccupied.

and their diffusion coefficients relative to each other, and it is proportional to the total number of receptors; the receptor occupancy (θ) at steady state is a function of the agonist concentration and the dissociation constant for that particular agonist (K_d). The efficiency (ϕ) is also agonist dependent. Thus the rate constant k_a contains factors that are specific to the agonist (K_d, ϕ) and a factor that is dependent on the agonist concentration (θ), as well as factors that are independent of the agonist (ω). The rate constant k_a is thus given by:

$$k_a = \omega\phi\theta = \kappa\theta \quad (4)$$

where κ combines factors that are independent of the agonist concentration. Substituting the expression for θ (eq. 1) into eq. 4 and then substituting this expression for k_a into eq. 3 and rearranging leads to the following equation:

$$f = \frac{\kappa}{\kappa + k_i} \frac{[A]}{[A] + \frac{k_i}{\kappa + k_i} K_d} \quad (5)$$

This equation and a comparison of this equation with the expression for f given in eq. 2 shows the following. 1) As [A] goes to ∞, f goes to $\kappa/(\kappa + k_i)$. That is, the maximum value for f at high agonist concentration (proportional to the intrinsic agonist V_{max}) is less than 1, because the ratio $\kappa/(\kappa + k_i)$ must be less than 1. If V_{max} for a given agonist is to approach the value corresponding to $f = 1$ (full activation), then the product of the variables that comprise κ must be significantly larger than k_i. According to this model the only difference in κ among agonists would be the difference in the efficiency fraction Ø, and a low value for Ø would result in partial agonism (when $f < 1$ even at high [A]) or, in the extreme case, complete antagonism (when Ø = 0). 2) EC_{50} equals $K_d\, k_i/(\kappa + k_i)$. This shows that the relationship between EC_{50} and K_d is also dependent on κ. For any $\kappa > 0$, the ratio $k_i/(\kappa + k_i)$ must be less than 1, and EC_{50} must then be less than K_d ($K_d/EC_{50} > 1$). Larger values for κ (such as would be due to high agonist efficiency) correspond to lesser values for the EC_{50}, relative to the K_d, and a higher K_d/EC_{50} ratio.

The first point demonstrates that the collision coupling model, with its assumption that the rate of cAMP generation is proportional to receptor occupancy, successfully predicts a "separation" between the response and binding curves as an essential aspect of such a serially linked system (i.e., it asserts that EC_{50} is necessarily less than K_d) (9). The second point demonstrates that the collision coupling model also relates the extent of the separation observed with a given agonist to the V_{max} for cyclase obtainable with that agonist. The predicted relationship between the EC_{50}/K_d ratio and the V_{max} is an accurate characterization of the relationship obtained in intact cells (2, 6).

Departures from the Predictions of Collision Coupling and an Explanation in Terms of a Modified Collision Coupling Model (the Encounter Coupling Model)

If, as according to collision coupling, the rate of G activation is strictly proportional to the concentration of agonist-bound receptors, then it should not matter whether that concentration is comprised of a small number of wholly occupied receptors or a larger number of receptors occupied only a fraction of the time. Experimental evidence indicates that for some agonists the latter case (where fractional occupancy is continuously redistributed and shared among a large receptor population) promotes a greater rate of activation than the first case, even if the concentration of agonist-bound receptors is identical (1, 2). Thus, there are certain circumstances for which the detailed predictions of the collision coupling model appear to be incorrect.

A simple explanation for the discrepancy between collision coupling and the results of such experiments is obtained if it is assumed that the interaction between any particular receptor and G occurs on some finite time scale that is at least as long as the time scale of the lifetime of an individual agonist-receptor complex (4). By assuming that the rate of G activation is proportional to receptor occupancy, it is an implicit assumption of the collision coupling model that the interaction (a "collision") is of zero duration. A collision of zero duration occurs with a receptor that is strictly either bound or unbound, such that for a fixed concentration of agonist-bound receptors each collision has the same probability of occurring with a receptor that is bound. Thus, if there is no opportunity for a receptor to change state in the course of a collision, because it is of zero duration, then the rate of G activation depends on the concentration of bound receptors no matter how rapidly (or slowly) the receptor occupancy is redistributed among the available number of receptors. If, instead, collisions occur in episodes of multiple collisions (an encounter), the duration of which is great enough that variation of the state of the receptor can occur during an encounter, then a potential explanation for the effect of occupancy redistribution

is obtained. In the case of encounters, all encounters are not necessarily equivalent with respect to agonist occupancy of receptors but depend on the lifetime of the agonist-receptor complex relative to the duration of an encounter.

The encounter coupling model is depicted schematically in Fig. 2. According to the model, a receptor arrives at an encounter with G in either the unbound or agonist-bound state. An encounter between this particular receptor and G pair lasts for some finite length of time, during which time the receptor may change state with respect to agonist occupancy. During any portion of an encounter when the receptor is occupied by agonist, an inactive G may become activated. The net rate of G activation depends on the duration of encounters, the frequency with which encounters occur, the lifetime of the receptor-agonist complex, and the overall receptor occupancy. The net activity of G and of cyclase depends on the net rate of G activation and on the receptor-independent rate of G inactivation.

This model makes only one assumption beyond the original collision coupling model, which is that an encounter between receptor and G takes place with a finite time of mean duration τ, with receptor and G effectively paired together during this time. A second assumption is that there is a fixed probability per unit time that G will become active while it is engaged in an encounter with an agonist-bound receptor (i.e., the activation of G is a first-order process during an encounter with an agonist-bound receptor). This is the simplest equivalent time-dependent analogue of the time-independent efficiency fraction that is a parameter in our description of collision coupling given above. The rate constant for G activation within one encounter when the agonist is bound is assumed to be agonist specific and is referred to as the efficiency, k_e, which value is presumed to reflect the relative ability of the agonist to select for an active conformation of the receptor (10).

To understand the essential likeness of collision coupling and encounter coupling, it is useful to consider two "boundary" cases for the relationship between receptor-agonist complex lifetimes and encounter times for which the encounter coupling system would behave according to the predictions of collision coupling. First, if the encounter time is small or instantaneous (i.e., if an encounter is indeed like a pure collision), then the opportunity for a change in state of the receptor is negligible during that time and the rate of activation of G is proportional to receptor occupancy. Second, if the mean lifetime of the receptor-agonist complex is so long, compared with the encounter time, that a change in state of the receptor during the encounter is unlikely, then the rate of activation of G is likewise proportional to the overall receptor occupancy. In the middle of these two boundary cases are circumstances for which the encounter coupling model predicts some dependence of the net rate of G activation on the agonist binding frequency. For instance, if G activation is achieved in only a fraction of the encounter time (i.e, if the receptor-agonist complex does not need a time as long as an encounter to ensure the activation of G), then a greater number of activations is achieved if the occupancy is distributed as rapidly as possible among all the receptors and among all ongoing encounters.

The potential effects of encounters as opposed to collisions are easily comprehended in qualitative terms. Below we examine the predictions of the encounter coupling model from a quantitative standpoint. We describe the calculation of the fractional adenylate cyclase activity, f, as a function of agonist concentration according to the encounter coupling model, taking into account the rate constants for agonist binding and unbinding, the efficiency of the agonist-bound receptor for activation of G during an encounter, the mean encounter time τ between receptor and G, and the frequency with which such encounters occur. We also describe the calculations for the case of agonist in competition with antagonist.

Calculation of G/Adenylate Cyclase Activity in Response to Agonist using the Encounter Coupling Model

Calculation of the net fractional G/adenylate cyclase activity obtained in the presence of a given concentration of agonist is derived in

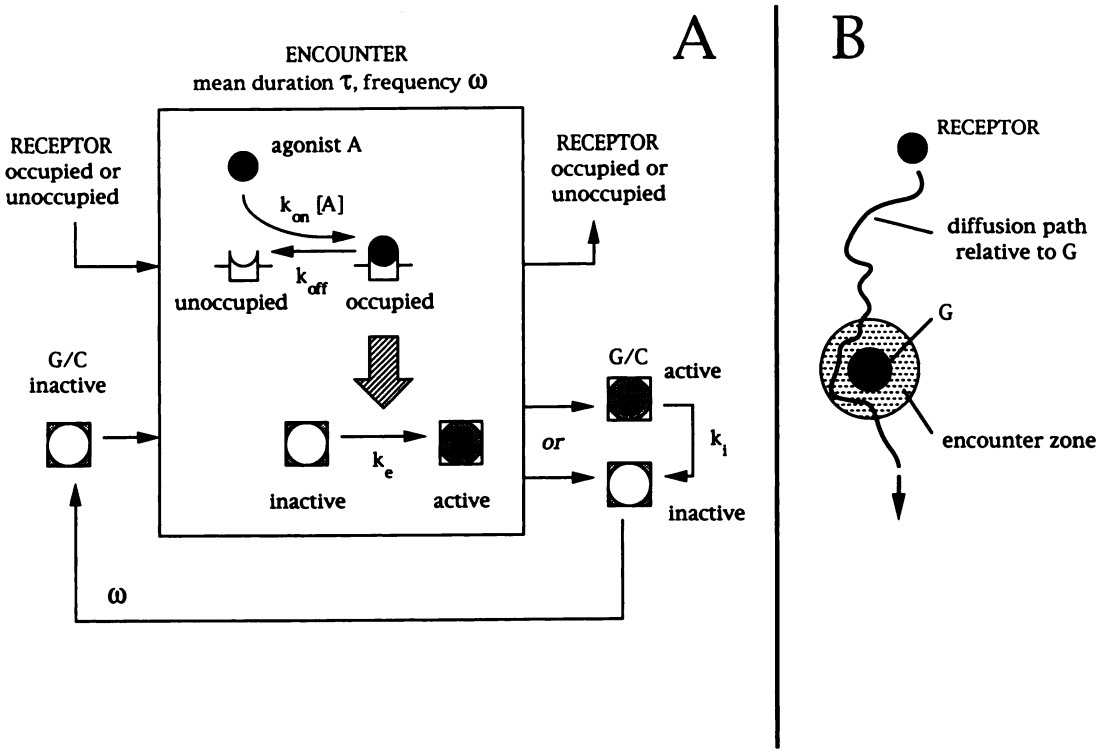

Fig. 2. Encounter coupling model. A, Activation and inactivation of G/cyclase (G/C). Activation of G/C occurs in the presence of receptor occupied by agonist during an encounter, with rate constant k_e. Inactivation of G/C is independent of receptor activity, as in the collision coupling model, with rate constant k_i. Encounters occur with frequency ω and with mean duration τ. B, Finite encounter duration. As opposed to a pure collision, an encounter between any one receptor and any one G takes place over some finite time, during which it is possible for the receptor to change state with respect to agonist occupancy. The rate of activation of G can, therefore, depend on the rate at which the receptor changes its occupancy state.

four steps using the encounter coupling model. First, the probability of activation of an inactive G in one encounter of a specific duration, t, is obtained. Second, the mean probability (P) of activation of an inactive G in one encounter of mean duration τ is obtained from the expression $P(t)$. Third, the overall rate constant for activation of G (k_a) is calculated given P and given the encounter frequency, ω. Fourth, the fractional activity f is calculated given k_a and given a value for the intrinsic G inactivation rate, k_i. We treat as equivalent the active state of G and the active state of cyclase, such that the steady state rate of cAMP generation (the response) is proportional to the fractional activity of G.

Probability of activation in one encounter of duration *t*. The encounter coupling model system for the calculation of the probability of activation of G during any one encounter of duration $t[P(t)]$ in the presence of a given concentration of agonist is shown schematically in Fig. 3. The probability of activation during an encounter is derived with the assumption that receptor and G remain in interactive proximity for the duration of an encounter. During an encounter the receptor can at any given instant be in one of two states, bound to agonist (a), or unbound (b). G can be in one of two states, inactive (c) or active (d). The inactive G can become activated ($c \rightarrow d$) in the presence of the agonist-bound receptor (b); this event is assumed to be first-order with respect to the presence of b, with rate constant (efficiency) k_e. The receptor can toggle between the bound and the unbound states ($a \leftrightarrow b$), irrespective of the state of G; the probabilities of these transitions are accounted for with the inclusion of the agonist concentration [A] and the rate constants for association (k_{on}) and dissociation (k_{off}).

The initial conditions for the calculation are the states of the receptor (a or b) and the state of G (inactive, c). The probability of activation in one encounter of duration t is the probability of transition of G from c (inactive) to d (active), in time t (the encounter duration), given the initial distribution of a and b according to the overall receptor occupancy and the rate constants for their interconversion. That is, given a receptor that arrives at an encounter with inactive G in either unbound or agonist-bound form, we calculate the probability that an encounter of duration t with an inactive G will result in an activation of G given the rate constant (efficiency) k_e. The system equations are:

$$\frac{da}{dt} = -k_{on}[A]a + k_{off}b \tag{6}$$

$$\frac{db}{dt} = k_{on}[A]a - (k_{off} + k_e)b \tag{7}$$

$$\frac{dd}{dt} = k_e b \tag{8}$$

where [A] is the agonist concentration.

The solution of interest is that for d and is of the form:

$$d(t) = D_1 e^{\lambda_1 t} + D_2 e^{\lambda_2 t} + D_0 \tag{9}$$

where λ_i are constants that depend on [A] and the rate constants k_{on}, k_{off}, and k_e, and where D_i are constants that depend on the initial conditions $a(0)$, $b(0)$, $c(0)$, and $d(0)$:

$$a(0) = \frac{K_d^A}{[A] + K_d^A} \tag{10}$$

$$b(0) = \frac{[A]}{[A] + K_d^A} \tag{11}$$

$$c(0) = 1 \tag{12}$$

$$d(0) = 0 \tag{13}$$

where $a(0)$ is the probability that a receptor is unoccupied upon entering an encounter and $b(0)$ is the probability that a receptor is occupied by

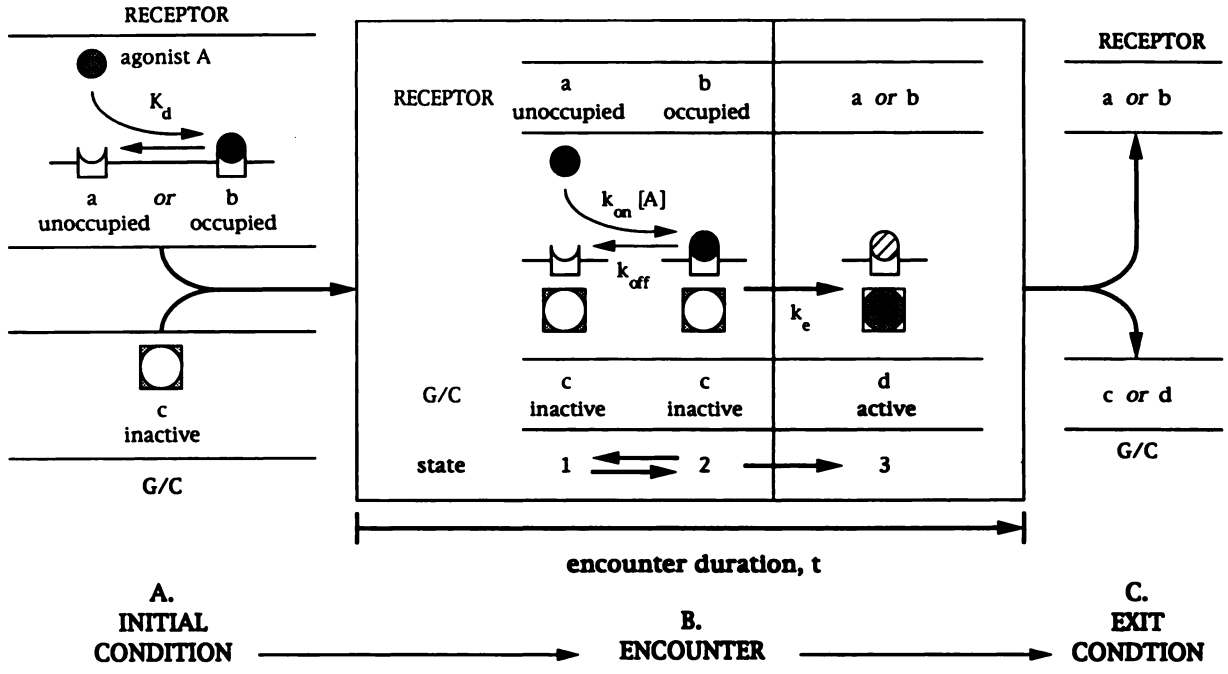

Fig. 3. Calculation of the probability of activation of G/cyclase (G/C) in one encounter of duration t. For the initial condition, the probability that the receptor is unoccupied (a) or occupied (b) is fixed according to the steady state occupancy (dependent on agonist concentration [A] and K_d), and G/C is inactive (c). During the encounter G/C can become active in the presence of agonist-occupied receptor, with an agonist-specific rate constant k_e (the efficiency); receptors can change occupancy states depending on [A] and the rate constants for association (k_{on}) and dissociation (k_{off}). Collectively, receptors and G/C can be in one of three states during an encounter, as shown, with possible transitions $1 \leftrightarrow 2 \rightarrow 3$. Although the exit from the encounter is shown from the *right side of the box* depicting the encounter, the exit can occur from each of the states 1, 2, or 3. The probability of activation in one encounter of duration t, $P(t)$, is given by the probability that the exit condition is in state 3 (containing species d, the active state of G/C). It is assumed that, on the time scale of any reasonable values for t, the probability of G/C inactivation is very small, and activation is, therefore, treated as an irreversible step.

agonist upon entering an encounter; $a(0) + b(0) = 1$. The complete analytical solution for $d(t)$ using complete expressions for D_i and λ_i are given in Appendix 1. The single value $d(t)$ $[0 < d(t) < 1]$ is a function of the initial conditions given above, the constants [A], k_{on}, k_{off}, K_d (k_{off}/k_{on}), and k_e (the assumed value for the efficiency), and the independent variable t.

It should be noted that the activation of G within a single encounter as modeled is irreversible. That is, we assume that any G activated during an encounter does not become inactive while the encounter persists. This is because a reasonable time scale for an encounter is anticipated to be small enough (on the order of 1 sec), compared with the mean inactivation time (at least 20 sec, given rate constant k_i = 1.0–3.0/min) (4), that the probability of this inactivation event is negligible in practical terms. Ignoring this improbable case makes this calculation decidedly more tractable. It should also be noted that the receptor is removed from the calculation, via species b (the agonist-bound receptor), with a rate equal to the rate of activation of G, even though the receptor is not "consumed" in the reaction *per se*. Because there can be no more than one activation for each encounter, the presence of the receptor after G activation is irrelevant, and the "removal" of the receptor forces the condition that this calculation yields at most one activation for each encounter. Thus, the probability of activation per encounter (which has a maximum value of unity) is not proportional simply to the mean residence time of receptors in state b during an encounter of duration t, as it would be if receptors were not removed via b at a rate equal to the rate of activation of G. Moreover, as the equations are written, the rate of conversion of species c (inactive G) to species d (active G) does not depend explicitly on c, because b exists only in the presence of c. In simpler terms, the calculation is of the probability of the transition in time t of the group composed of receptor (a or b) and G (c or d) to the state labeled 3 in Fig. 3 [(a or b) + d], where the initial distribution is between states 1 ($a + c$) and 2 ($b + c$) and the possible interconversions are $1 \leftrightarrow 2 \rightarrow 3$.

Mean probability of activation in one encounter of mean duration τ. Above, we calculated the probability of activation in one encounter, $P(t)$, for an encounter that has a fixed duration, t. It is unrealistic, however, to model all encounters using a single fixed value for the encounter duration. Instead, we will allow for a distribution in the duration of encounters, where the distribution has some fixed mean lifetime, τ. Given this distribution we calculate the mean probability of activation for encounters with mean lifetime τ using the expression for $P(t)$.

The appropriate distribution of encounter times is given by an exponential decay function for the lifetime (or probability of "survival") of an encounter. That is, we treat an encounter as a process that, once begun, has a fixed probability per unit time that it will end. Thus

$$\text{probability of survival} = e^{-k_s t} \tag{14}$$

where k_s is the rate constant for the survival of encounters. If the mean lifetime of encounters is τ, then $k_s = 1/\tau$. This simple characterization of the lifetime of an encounter is in keeping with expectations based either on simple diffusion of two molecules or on specific receptor/G interactions.

In order to account for the distribution of encounter duration, the mean probability of activation in one encounter, P, can be obtained by a weighted summation of the probabilities for all encounters of fixed duration, $P(t)$, where the weighting factor is related to the probability that a given encounter will have that fixed duration. This sum can be approximated by the sum of weighted probabilities for an infinite number of adjacent time intervals of width δt, with a center value of t:

$$P = \sum_{t=0}^{t=\infty} P(t)[e^{-k_s(t-\delta t)} - e^{-k_s(t+\delta t)}] \tag{15}$$

That is, by treating a single $P(t)$ as an approximate $P(t)$ for the interval $(t - \delta t)$ to $(t + \delta t)$, the specific contribution of P of encounters having duration t is given by a single term in the summation. Analytically, this summation expression is replaced by the integral

$$P = \int_0^\infty P(t) \frac{-d(e^{-k_s t})}{dt}\, dt \tag{16}$$

or

$$P = k_s \int_0^\infty P(t) e^{-k_s t}\, dt \tag{17}$$

Given eq. 9 for $P(t)$, then

$$P = D_1 \frac{k_s}{k_s - \lambda_1} + D_2 \frac{k_s}{k_s - \lambda_2} + D_0 \tag{18}$$

where $k_s = \tau^{-1}$, for a mean encounter duration of τ.

Rate constant for G activation, k_a. Given the probability of activation of one encounter, P, the rate constant for G activation, k_a, is calculated given the encounter frequency ω (the number of encounters per unit time). Using a distribution of encounter frequencies with mean value ω, the rate constant k_a is simply the product of P and the encounter frequency ω:

$$k_a = P\omega \tag{19}$$

The derivation of this result is shown in **Appendix 2.**

Calculation of the fractional cyclase activity, f. The fractional cyclase activity f is then calculated from k_a given the cyclase inactivation rate, k_i:

$$f = \frac{k_a}{k_a + k_i} \tag{20}$$

In summary, the calculation of f depends on the distribution of receptor occupancy by agonist (which fixes the initial conditions for a receptor entering an encounter), the rate constants for binding and unbinding of agonist (to account for the possible effect of "switching" of the state of the receptor, during an encounter, between being bound to agonist and being unbound), and the efficiency, k_e, of the agonist-bound receptor to activate G. Given the calculated probability of activation in one encounter of mean duration τ, the overall rate constant for activation (k_a) is calculated given the mean frequency of encounters, ω (previously estimated to be $0 < \omega < 100$ min^{-1}) (4), and the fractional activity of cyclase, f, is calculated given the inactivation rate constant, k_i (previously estimated to be between 0.5 and 3 min^{-1}) (3). The initial conditions for the encounter depend on the measured values for K_d (2 μM for epinephrine) (11); the value for the association rate constant has been deduced to be on the order of 4×10^8 M^{-1} min^{-1} (3).

Receptor Mobility-Limited Agonist Concentration versus Response Curve

For comparison with the normal agonist concentration versus cyclase response curve given above, we also compute the response curve predicted by the encounter coupling model for the case in which agonist binding frequency is removed as a contributing component (4). The activation of G for the receptor mobility-limited case is one in which the activation rate constant k_a is obtained via the activity of some number n of receptors that are occupied 100% of the time. In comparison with the normal concentration versus response curve, the receptor mobility-limited concentration versus response curve is that obtained when the number of bound receptors is given by $n = \theta N$, where N is the total number of receptors and θ is the occupancy at any point on the normal concentration versus response curve. That is, to obtain the receptor mobility-limited concentration versus response curve, the N receptors occupied some fraction θ of the time (where θ is a function of the agonist concentration) in the normal concentration versus response curve are replaced by a specific fraction of that number of receptors (θN) occupied 100% of the time.

The calculation of k_a for this special circumstance is straightforward (4). The calculation of $P(t)$ for an encounter with one fully occupied receptor is given by

$$P(t) = 1 - e^{-k_e t} \tag{21}$$

For a mean encounter duration τ, P is then given by

$$P = \frac{k_e}{k_e + k_a} \tag{22}$$

Because only a fraction θ of the total number of encounters can have occupied receptors (by definition for this special case), the effective encounter frequency is reduced from ω to $\theta\omega$, and k_a is given by

$$k_a = P\theta\omega \tag{23}$$

where θ is calculated at a given concentration of agonist, [A], given K_d ($\theta = [A]/[A] + K_d$). The fractional activation f is computed as before (eq. 20) given k_i, $f = k_a/(k_a + k_i)$.

Calculation of Cyclase Activation in Response to Agonist in the Presence of a Competing Antagonist

The encounter coupling model for a system with competitive binding of receptors by agonist and antagonist is shown in Fig. 4. In this system the receptor can be in one of three states within an encounter, i.e., antagonist bound (h), unbound (a), or agonist bound (b) ($h \leftrightarrow a \leftrightarrow b$). As for the agonist-only case, G can make the transition from the inactive state (c) to the active state (d) ($c \rightarrow d$), with a probability per unit time that is proportional to the presence of agonist-bound receptor, b. Again, in this case b is removed from consideration in any one encounter once G has become activated. The system equations are:

$$\frac{dh}{dt} = -k_{off}^{B}h + k_{on}^{B}[B]a \tag{24}$$

$$\frac{da}{dt} = k_{off}^{B}h - (k_{on}^{A}[A] + k_{on}^{B}[B])a + k_{off}^{A}b \tag{25}$$

$$\frac{db}{dt} = k_{on}^{A}[A]a - (k_{off}^{A} + k_e)b \tag{26}$$

$$\frac{dd}{dt} = k_e b \tag{27}$$

where [A] and [B] are the agonist and antagonist concentrations, respectively. The solution of interest is that for d and is of the form:

$$d(t) = D_1e^{\lambda_1 t} + D_2e^{\lambda_2 t} + D_3e^{\lambda_3 t} + D_0 \tag{28}$$

where λ_i are constants that depend on [A], [B], and the different rate constants k and where D_i are constants dependent on the initial conditions $h(0)$, $a(0)$, $b(0)$, $c(0)$, and $d(0)$:

$$h(0) = \frac{\frac{[B]}{K_d^B}}{1 + \frac{[A]}{K_d^A} + \frac{[B]}{K_d^B}} \tag{29}$$

$$a(0) = \frac{1}{1 + \frac{[A]}{K_d^A} + \frac{[B]}{K_d^B}} \tag{30}$$

$$b(0) = \frac{\frac{[A]}{K_d^A}}{1 + \frac{[A]}{K_d^A} + \frac{[B]}{K_d^B}} \tag{31}$$

$$c(0) = 1 \tag{32}$$

$$d(0) = 0 \tag{33}$$

where, $h(0)$ is the probability that a receptor is occupied by antagonist upon entering an encounter, $a(0)$ is the probability that a receptor is unoccupied upon entering an encounter, and $b(0)$ is the probability that a receptor is occupied by agonist upon entering an encounter [$h(0) + a(0) + b(0) = 1$]. The complete analytical solution for $d(t)$ for the agonist plus antagonist case is also given in Appendix 1. In analogy to the agonist-only case, the calculation of $d(t)$ for this case is of the probability of the transition in time t of the group composed of receptor (h, a, or b) and G (c or d) to the state labeled 3 in Fig. 4 [(h or a or b) + d], where the initial distribution is between states 4 ($h + c$), 1 ($a + c$), and 2 ($b + c$) and the possible interconversions are $4 \leftrightarrow 1 \leftrightarrow 2 \rightarrow 3$.

Following the derivation for the agonist-only case given above, the

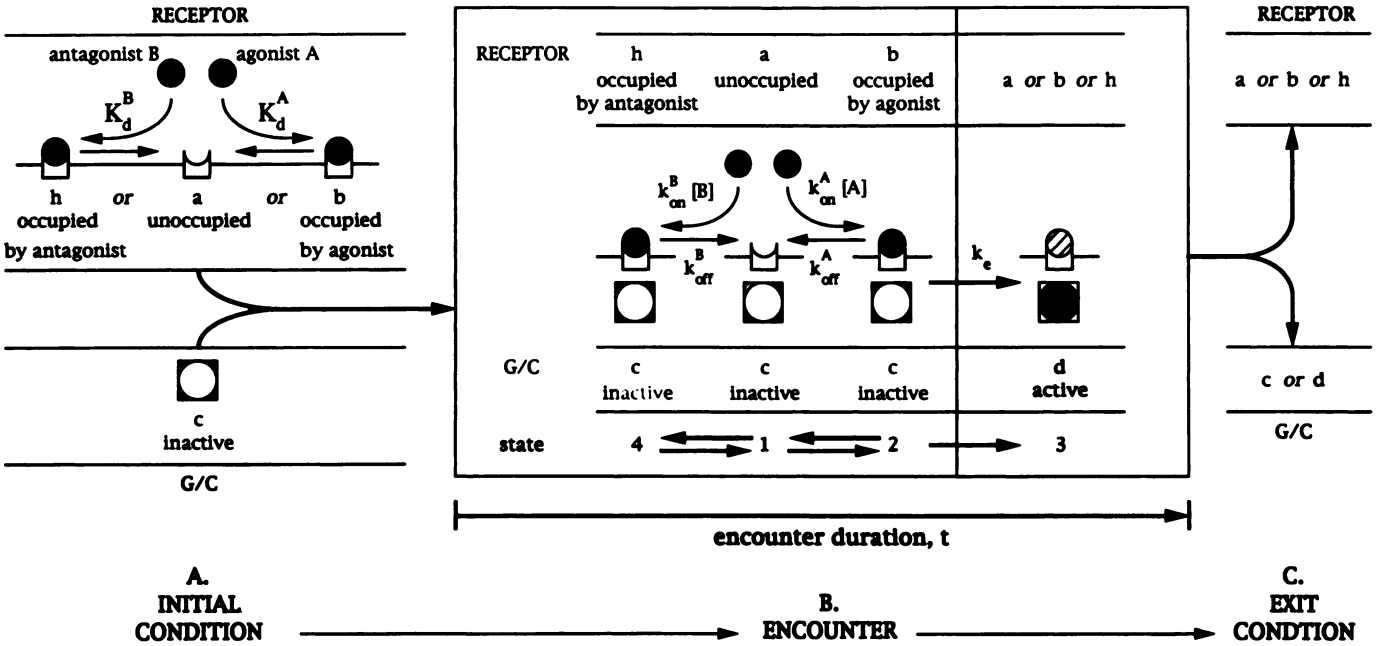

Fig. 4. Calculation of the probability of activation of G/cyclase (G/C) in one encounter of duration t in the presence of competing agonist and antagonist. The initial condition includes receptor occupied by antagonist (h) and depends on the concentration of antagonist, [B], and agonist, [A], and their dissociation constants (K_d^A and K_d^B). The possible collective states of receptor and G/C during an encounter include one with the antagonist-occupied receptor (state 4), with possible transitions $4 \leftrightarrow 1 \leftrightarrow 2 \rightarrow 3$. Transitions between states of agonist and antagonist occupancy are dependent on [A] and [B] and the rate constants for association and dissociation (k_{on}^A, k_{off}^A, k_{on}^B, and k_{off}^B). As for the agonist-only case (Fig. 3), $P(t)$ is given by the probability that the exit condition is in state 3 (containing species d, the active state of G/C).

mean probability of activation in one encounter with mean value τ for this case is given by:

$$P = D_1 \frac{k_a}{k_a - \lambda_1} + D_2 \frac{k_a}{k_a - \lambda_2} + D_3 \frac{k_a}{k_a - \lambda_3} + D_0 \qquad (34)$$

where $k_a = \tau^{-1}$.

For the case of agonist A and antagonist B, the single value P, the probability of activation in one encounter of mean duration τ, is calculated for a fixed combination of [A] and [B], given k_{on}^A, k_{off}^A, K_d^A (k_{off}^A/k_{off}^A), k_{on}^B, k_{off}^B, K_d^B (k_{off}^B/k_{on}^B), k_e (the efficiency) and τ. Then, as for the agonist-only case, f is calculated given P, ω, and k_i.

Results

Agonist concentration versus response curve for epinephrine calculated using the encounter coupling model. The approach to the calculation of an agonist concentration versus cyclase response curve is to find a combination of variables k_e, ω, τ, and k_i such that the appropriate fractional activation f is obtained at some single agonist concentration [A], where f is given by $[A]/([A] + EC_{50}) = k_a/(k_a + k_i)$. For instance, at an agonist concentration [A] equal to the EC_{50}, $f = 0.5$ and $k_a = k_i$. For the case [A] = EC_{50}, the receptor occupancy, $\theta = EC_{50}/(EC_{50} + K_d)$, forms the initial condition for the calculation of P.

An example of combinations of k_e and τ that result in an EC_{50} equal to the EC_{50} for epinephrine (10 nM) is shown in Fig. 5 for a series of values for ω, with fixed values for K_d (2 μM), k_{on} (4×10^8 M^{-1} min^{-1}), and k_i (1.5 min^{-1}). *A priori*, an infinite number of combinations of values for k_e and τ will result in a given EC_{50} given values for K_d, k_{on}, ω, and k_i. In this example we have used measured values or used approximate values deduced for these constants (1, 3, 4). The encounter frequency ω, for instance, has been shown to be in the range of 20–100 min^{-1}, which range of values is used as a parameter in Fig. 5. According to the model calculations, increases in τ decrease the value of k_e needed to obtain a sufficient fraction of successful encounters that result in $k_a = k_i$. Similarly, increases in ω decrease the value of k_e needed to obtain the same k_a.

The curves of k_e versus τ shown in Fig. 5 were based on a calculation at [A] = EC_{50}. In addition, the values for k_e and τ at any one point in the curves in Fig. 5 also reproduce a normal dose-response curve across the entire range of agonist concentrations using the given values for ω and k_i. That is, the encounter coupling model calculations using a given set of constants from Fig. 5 reproduce an agonist concentration versus cyclase response curve that is indistinguishable in practical terms from a hyperbola characterized by the EC_{50}, $f = [A]/([A] + EC_{50})$. This is demonstrated by an example concentration versus response curve for epinephrine shown in Fig. 6. An EC_{50} of 10 nM is obtained using the values $\tau = 1.5$ sec, $k_e = 6.9$ sec^{-1}, $\omega = 45$ min^{-1}, and $k_i = 1.5$ min^{-1}.

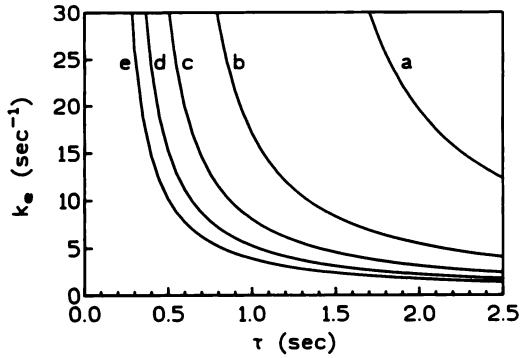

Fig. 5. Calculated values of k_e versus τ for receptor-mediated activation of G by epinephrine (K_d = 2 μM, EC_{50} = 10 nM) according to the encounter coupling model (using eq. 20, from preceding eqs. 6–19). Plots are shown for a series of values for the encounter frequency ω (*a*, 20 min^{-1}; *b*, 40 min^{-1}; *c*, 60 min^{-1}; *d*, 80 min^{-1}; *e*, 100 min^{-1}).

Although the mean encounter duration, τ, is not known explicitly, an estimate of τ = 1.5 sec can be made on the following basis. The mean lifetime of the epinephrine-receptor complex has been estimated to be on the order of 0.25 sec (3). The forced diminution of the contribution of binding frequency can reduce k_a by a factor of approximately 6 (1). Therefore, it is plausible that the mean encounter time is a factor of 6 times the mean epinephrine lifetime, because the unrestricted rate of redistribution of epinephrine occupancy among all available receptors results in a 6-fold increase in the rate of activation, compared with the case in which occupancy turnover is restricted.

The predicted effects of such a restriction of occupancy turnover are shown in Fig. 6 by a comparison of the receptor mobility-limited concentration versus response curve and the normal concentration versus response curve. As described under Theory, for the receptor mobility-limited concentration versus response curve the activity of N receptors occupied a fraction θ of the time for the normal concentration versus response curve is replaced by that of $N\theta$ receptors occupied continuously. Using the example set of parameters given in Fig. 6, the effect of the elimination of the turnover occupancy is to shift the concentration versus response curve to the right of the normal concentration versus response curve. That is, for this case a given fractional activation is obtained for the receptor mobility-limited case at a concentration of agonist (and a concentration of agonist-bound receptors) that is greater than the concentration that would be necessary if the turnover of receptor occupancy was not restricted. The example value of ω = 45 min^{-1} in Fig. 6 was chosen on the basis of shifting the EC_{50} from $K_d/200$ (R = 200) for the normal concentration

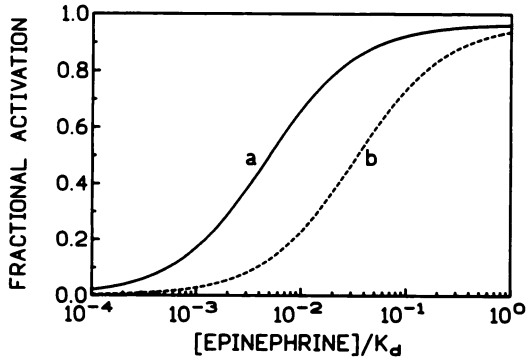

Fig. 6. Calculated agonist concentration versus cyclase response curves using the encounter coupling model. *a*, Theoretical epinephrine concentration versus cyclase response curve calculated using the encounter coupling model, giving EC_{50} = 10 nM (using eq. 20 and preceding eqs. 6–19). Input values were k_e = 6.9 sec^{-1}, τ = 1.5 sec, ω = 45 min^{-1}, k_i = 1.5 min^{-1}, K_d = 2 μM, and k_{on} = 4×10^8 M^{-1} min^{-1}. *b*, Theoretical epinephrine concentration versus cyclase response curve calculated using the encounter coupling model for the case in which the turnover of epinephrine occupancy among the receptor population is eliminated (the receptor mobility-limited concentration versus response curve, using eq. 20, with k_e calculated using eqs. 21–23). Input values were the same as in *a*, with the exception of the unneeded value for k_{on}.

versus response curve to $K_d/30$ ($R = 30$) for the receptor mobility-limited case, as has been observed experimentally (1).

Predicted effects of increasing the agonist binding frequency. A prediction of the encounter coupling model is that the mean lifetime of the receptor-agonist complex can influence the net observed activity of a given concentration of agonist-bound receptors. In Fig. 7, the results of encounter coupling model calculations are shown in which the constants employed in Fig. 6 (an example based on constants assumed for epinephrine) are used but the lifetime of the receptor-agonist complex is varied. The lifetime of the receptor-agonist complex is varied by assuming a fixed rate constant for association (k_{on}) and varying K_d, such that changes in the dissociation constant are reflected solely in the dissociation rate constant, k_{off}. As shown in Fig. 7, the EC_{50} values of concentration versus response curves can vary between two limits, relative to the K_d. At low K_d (long agonist-receptor lifetimes), the concentration versus response curve approaches the condition of the receptor mobility-limited curve, in which the activation rate cannot exceed the value of $\theta\omega$, and the K_d/EC_{50} ratio approaches the same limit as that for the receptor diffusion-limited case shown in Fig. 6. At increasingly higher K_d (shorter agonist-receptor lifetimes), the activation rate constant k_a cannot exceed the value of ω even if k_e is essentially infinite; using $k_e = 6.9\ sec^{-1}$ as in Fig. 6, the K_d/EC_{50} ratio for the infinitely short receptor-agonist lifetime approaches a limit that is only slightly greater than that for the ratio calculated for the epinephrine-based example.

Effects of variation of k_e, τ, ω, and k_i on K_d/EC_{50}. Using the constants employed in Fig. 6 as a basis case, the effects on the K_d/EC_{50} ratio of variation of k_e, τ, ω, and k_i are shown in Fig. 8. With all other factors held constant, increases in k_e, τ, or ω can increase the calculated K_d/EC_{50} ratio, whereas an increase in k_i decreases the calculated K_d/EC_{50} ratio. Because the basis case is used only as an example, these results are best understood in purely general terms. Increases in k_e, τ, or ω increase the net activity of G per extent of receptor occupancy, thereby increasing the K_d/EC_{50} ratio. An increase in the inactivation rate constant k_i decreases the net G activity obtained at any particular degree of receptor occupancy, thereby decreasing the K_d/EC_{50} ratio.

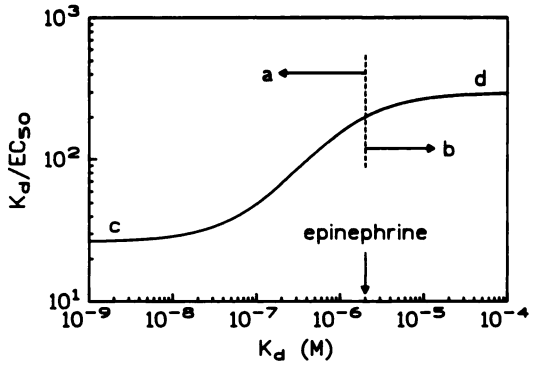

Fig. 7. Effect of variation of the receptor-agonist complex lifetime on the separation (K_d/EC_{50} ratio) between binding and response curves calculated using the encounter coupling model. The curve was calculated using eq. 20 and preceding equations for values of $k_e = 6.9\ sec^{-1}$, $\tau = 1.5$ sec, $\omega = 45\ min^{-1}$, $k_i = 1.5\ min^{-1}$, and $k_{on} = 4 \times 10^8\ M^{-1}\ min^{-1}$. k_{off} was varied to obtain K_d (k_{off}/k_{on}) from 10^{-9} M to 10^{-4} M. Fractional activation f was calculated as a function of [agonist] using eq. 20; EC_{50} was obtained (by definition) by EC_{50} = [agonist] when $f = 0.5$. The ratio K_d/EC_{50} was calculated and plotted versus K_d. Varying only k_{off} alters only the mean receptor-agonist lifetime, t_m (related to k_{off} by $t_m = k_{off}^{-1}$); compared with epinephrine ($K_d = 2\ \mu M$), a lower K_d (in the direction of *arrow a*) reflects a longer t_m, whereas a higher K_d (in the direction of *arrow b*) reflects a shorter t_m. Using the constants given above, variation of only k_{off} causes the ratio K_d/EC_{50} to range between limits of the K_d/EC_{50} ratio observed for the receptor mobility-limited response curve (approximately 30) (*c*) and a ratio that is only marginally greater than that observed for the normal epinephrine response curve (approximately 200) (*d*).

Comparison of the predictions of the encounter coupling model with previous experimental results for competitive antagonism. In experiments reported previously (1), adenylate cyclase activation was measured for a constant concentration of epinephrine-bound receptors in the presence of increasing concentrations of antagonist, to test for the potential effect of epinephrine binding frequency to influence the cyclase activation rate. If the rate of G activation is strictly proportional to receptor occupancy (with no contribution of binding frequency), then the effect of the presence of an antagonist should be an effective increase in the agonist EC_{50}, according to the Cheng-Prusoff relation (2, 12):

$$f = \frac{[A]}{[A] + \left(1 + \frac{[I]}{K_I}\right)EC_{50}} \qquad (35)$$

In the experiments, both [A] and [I] were increased in order to keep the right side of the equation constant and equal to f for the control case in which no antagonist I was present. Using an antagonist that is presumed to have a short receptor complex lifetime (metoprolol), there was no effect of changing both [A] and [I] together. In contrast to this result, the use of an antagonist with a long receptor complex lifetime (propranolol) caused a significant decrease in f under the same protocol.

Because of the long lifetime of the propranolol-receptor complex, a condition was approached in the presence of high concentrations of propranolol in which the control concentration of epinephrine-bound receptors was obtained with a fraction of the receptors occupied most of the time with epinephrine (in contrast to the control case, in which epinephrine occupied all of the available receptors for some average fraction of the time), and the cyclase activation rate approached that for the receptor mobility-limited case.

This experiment can be modeled by the encounter coupling model equations given for the presence of a competing antagonist. The effects of the addition of antagonist predicted using the encounter coupling model are shown in Fig. 9 for two different antagonists. For the calculated curves, the only difference between the two antagonists was assumed to be the mean lifetime of the receptor-antagonist complex (k_{off}^{-1}), whereas the association rate constants were assumed to be equal to that of propranolol ($k_{on} = 4 \times 10^{-8}\ M^{-1}\ min^{-1}$) (1, 3). That is to say, the different affinities of the two antagonists (propranolol K_d = 650 pM, metoprolol K_d = 240 nM) were presumed to be due only to differences in their dissociation rate constants, k_{off}. For the propranolol experiment, the model predicts a progressive and significant decrease in adenylate cyclase activity as both epinephrine and propranolol are increased, in parallel with the experimental results. For the metoprolol experiment, the model predicts no change in adenylate cyclase activation, as was observed in the experiment.

In the ideal circumstance, both of the experimental data curves in Fig. 9 would produce a fractional activity for the

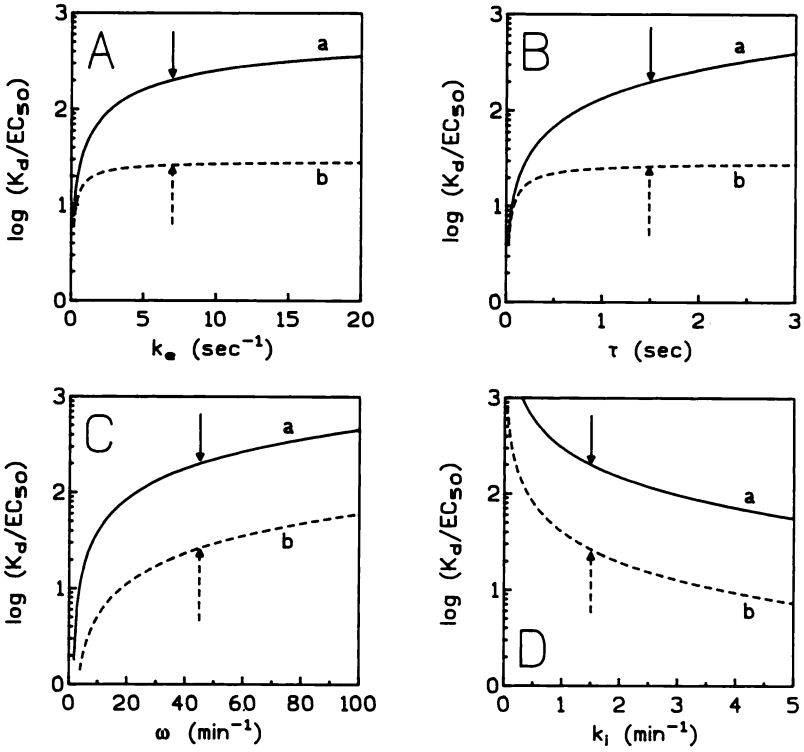

Fig. 8. Effect of variation of k_e, τ, ω, and k_i on the separation between binding and response curves calculated using the encounter coupling model. A, Effect of variation of efficiency, k_e. B, Effect of variation of encounter duration, τ. C, Effect of variation of encounter frequency, ω. D, Effect of variation of the G/C inactivation rate constant, k_i. In A-D, the *solid line* (*a*) indicates the K_d/EC_{50} ratio calculated for the normal concentration versus response curve with unrestricted turnover of agonist occupancy among the receptor population (using eq. 20); the *dashed line* (*b*) indicates the K_d/EC_{50} ratio calculated for the receptor mobility-limited case (using eq. 20, but with k_e given by eq. 23). In each case, the basis set of values for the encounter coupling model parameters were set to those used in Fig. 6, and *arrows* indicate the points corresponding to the ratio K_d/EC_{50} for the basis set (from *curves a* and *b* in Fig. 6).

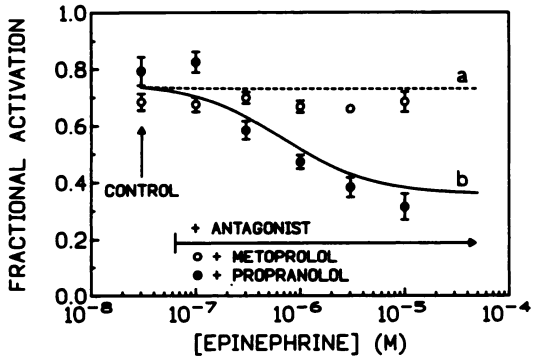

Fig. 9. Adenylate cyclase activity in response to a constant concentration of epinephrine-bound receptors but in the presence of different concentrations of antagonist. Data shown are from experiments reported previously (1). A constant concentration of epinephrine-bound receptors equal to control (without antagonist) was achieved in the presence of increasing epinephrine concentrations by increasing the concentration of a competing antagonist [propranolol (●) or metoprolol (○)] (antagonist concentrations not shown). Calculated response using the encounter coupling model for the agonist plus antagonist case (based on eq. 34): (*a*), K_d^B = 240 nM (metoprolol); (*b*), K_d^B = 650 pM (propranolol); other constants were the same as those used for the calculations shown in Fig. 6, with $k_{on}^B = k_{on}^A$. For each *point*, the antagonist concentration [B] is given by

$$[B] = K_d^B\left(\frac{[A]}{K_d^A}\frac{1}{\theta} - \frac{[A]}{K_d^A} - 1\right) \quad (36)$$

where K_d^A is K_d for epinephrine (2 μM) and θ is the epinephrine occupancy for the control point [[A] = 3×10^{-8}, θ = [A]/([A] + K_d^A) = 0.015].

control case that is exactly equal to 0.75; however, each of the data curves was slightly offset from the idealized case when the measured cAMP accumulations were normalized to the maximum cAMP accumulations that were obtained for each experiment (1). Nonetheless, the distinction between the results obtained with the two antagonists is clear, and the trends of the predictions of the encounter coupling model correspond to the contrasting experimental results using the two agonists. Whether or not the constants assumed for k_e, τ, ω, and k_i are either accurate or precise (they are the same as those used in the calculations for the example concentration versus response curve shown in Fig. 6), it is clear that the encounter coupling model is consistent with the difference effects of different antagonists on this system, which could be explained by differences in the receptor-antagonist complex lifetime.

Discussion

Recent investigations into the relationship between receptor occupancy and the rate of activation of G/adenylate cyclase have shown that the rate of activation can be dependent in part on the rate of turnover of agonist occupancy among the receptor population. This result suggested that the interaction between single receptor and G pairs may take place on a time scale that is longer than the mean lifetime of the epinephrine-receptor complex, possibly due to multiple collisions between them rather than a single isolated collision. Here we have formulated the kinetics of G activation in terms of the encounter coupling model in order to examine whether the detailed model can account for the demonstrated effects of agonist binding frequency on activation rate. The encounter coupling model is an extension of the collision coupling model, in that it builds on the established premise that any one G can be accessed by more than one receptor. According to the encounter coupling model, the interaction between individual receptors and G has a finite duration. Because of this duration, the agonist binding frequency is, according to the encounter coupling model, a factor that affects the cyclase response to agonist. In the encounter coupling model, the rate constants for agonist association and dissociation are taken into account, as well as the encounter

frequency, encounter duration, and an agonist-specific efficiency for activation (for a receptor bound to agonist and within an encounter). This model was shown above to give results consistent with the normal agonist concentration versus response curve, as well as the results of experiments in which the access of agonist to receptors was altered by the addition of antagonist.

A number of constants were used in the calculations of the predictions of the encounter coupling model. It is important to note, however, that only the numerical values for τ and k_e are essentially unknown. The remainder of the constants (the first-order rate constant for dissociation, k_{off}, the second-order rate constants for association, k_{on}, the dissociation constants, K_d, the encounter frequency, ω, and the inactivation rate constant, k_i) have been measured or are known by deduction within reasonable limits. Thus, although the calculation might appear at first glance to be overly malleable on the basis of the number of input values, the restrictions are in reality fairly tight. The result to be emphasized is simply that the encounter coupling model provides predictions for the extent of adenylate cyclase activation that are both qualitatively and quantitatively consistent with data that cannot be explained by the collision coupling model without the modifications that account for an encounter of non-zero duration. The analysis itself is not a form of evidence for the truthfulness of the model representation of the interactions between agonist, receptor, and G but is, rather, an interpretation of the data from which it was derived and with which it appears to be consistent. This consistency is demonstrated using only "round" numbers for the input constants, without any elaboration via curve fitting or multiparameter analyses. That is to say, it is not necessary to seek out any special combination of values for constants in order to show the general consistency of the model with the data. Thus, our point is not to assert that the system must behave as modeled or that the values of the constants used in the examples must reflect precise values. Instead, we assert only that the predictions of the model are in accordance with phenomena that have been observed experimentally.

In the examples given, we have used an estimate of the mean encounter duration equal to 1.5 sec. This estimate for the encounter duration was based on previous results demonstrating and quantitating the contribution of epinephrine binding frequency to the epinephrine concentration versus response curve. Those results were that binding frequency contributed up to a 6-fold increase in the rate of cyclase activation in the steep region of the dose-response curve (Fig. 3 in Ref. 1). Considering that the lifetime of the epinephrine-receptor complex is on the order of 0.25 sec (3), then in the context of the model it is expected that the encounter duration must be at least 6 times as great. With this order of magnitude of the encounter duration, a receptor can change occupancy states in the presence of epinephrine during the course of an encounter. Because the epinephrine-bound receptor is efficient at G activation and the epinephrine binding frequency is high, departures from the collision coupling model are observed under conditions of high occupancy by epinephrine of a small receptor number. Similar results that are consistent with this interpretation have been obtained for isoproterenol and metaproterenol stimulation of adenylate cyclase in intact S49 cells (2).

The exact nature of an encounter between receptor and G of this estimated duration is unknown. It is therefore important to examine the estimate for the encounter duration in terms of the diffusion of receptors and G in the cell membrane. It is reasonable to expect that the mean free path of diffusion in the membrane would be short and that two molecules that collide once are likely to collide more than once a number of times in sequence. However, even given a rather low diffusion coefficient ($0.01\ cm^2\ s^{-1}$), a calculation of the expected lifetime for such a sequence of collisions (i.e., the mean period of time that two molecules would remain in interactive proximity when the radius of interaction is on the order of the size of the molecules) is much shorter (on the order of 10 msec) than the encounter duration used here (results not shown). The encounter frequency, on the other hand, is consistent with a diffusion coefficient on the order of $0.1\ cm^2\ sec^{-1}$ (1, 4). This order of magnitude for the diffusion coefficient is in turn consistent with measurements of G $\beta\gamma$ subunit mobility made in NG-108-15 cells by Kwon *et al.* (13). Thus, it is possible that the diffusion of receptor and G relative to each other is reduced once they are within an encounter; the net diffusion of receptors over longer periods may be composed of a combination of periods during which diffusion is relatively uninhibited, interspersed with periods during which diffusion is relatively restricted.

A priori, the long association time between receptor and G could be the result of the formation of a specific complex with specific orientations of the two molecules. This possibility has been eliminated by Tolkovsky et al. (14) for turkey erythrocyte membrane preparations. In those experiments they showed that the lifetime of the receptor-G complex was very short, compared with the time required for activation of G. It is possible that in the intact S49 cell system the detailed kinetics might be sufficiently different for there to be a different rate-determining step. However, it seems more likely that the tendency of membrane proteins to form aggregates within the plane of the membrane plays a major role. This would be consistent with the turkey erythrocyte data. In physical terms an encounter pair would be a molecular aggregate held together by surface energies rather than aligned and oriented forces that would maintain the molecules in a complex with a well defined geometry. The presence of such aggregates is almost inevitable; the question is whether their lifetimes are such as to account for the long encounter times that are necessary to explain the present data via the encounter coupling analysis. However, it may be easily supposed that once two large molecules are juxtaposed in the bilayer it might require a significant activation energy to separate them. There is a good deal of evidence that many membrane proteins form two-dimensional aggregates within the membrane. The idea of short-lived aggregates of two or more membrane proteins is equivalent to the standard physical concept of the nonideal solution. Even in the more normal three-dimensional solution the colligative properties of most proteins only approach ideality at very high dilution. Such is clearly not the case where a large number of protein molecules are packed together within the plane of the cell membrane. Thus, although simple diffusion cannot explain such long encounter times, the contribution of nonspecific forces, leading to "stickiness" between two protein molecules, may be a sufficient explanation.

With respect to the estimated duration of encounters, it is useful to comment on the fidelity of receptors to G during an encounter. It is important to note that the experimental data

provide no evidence that an encounter between one receptor and G pair occurs only with the exclusion of encounters of that same receptor with some other G or vice versa. In other words, the fidelity of a receptor to G with which it is engaged may not be absolute, and there is no basis to regard an encounter as an oriented quasistable interaction. The encounter coupling model likewise does not require that encounters between receptors and G be exclusive events.

In the formulation for encounter coupling given here we have assumed that the relationship between receptor and G/cyclase during an encounter is not the "ternary complex" where there is cooperative binding between agonist and G on the receptor. We have assumed [with Tolkovsky *et al.* (14)] that in the presence of GTP the ternary complex is short lived and that it does not significantly affect the overall measured estimate of K_d for agonist of the receptor in intact cells (2).

Some other implicit assumptions of the encounter coupling model calculations should be noted. It was assumed that the agonist binding is independent of encounters, that the association rate constants for all ligands (agonist and antagonists) are equal, and that the encounter duration τ is not dependent on the presence or identity of an associated ligand. Although these assumptions may not be true when considered at the finest level of detail, the predictions of the encounter coupling model using these assumptions are nonetheless consistent with the experiments. We have made no particular assumptions with respect to receptor interactions with G, relative to G subunit dissociation and GTPase activity. Both the encounter coupling and collision coupling models assume that the transducing capability of an active G is relatively long-lived after activation, whatever are the molecular details of the mechanism whereby the G is reset, by subunit reassembly or GTP hydrolysis or both. In both models the interaction of a receptor with active G has no effect; it does not influence the rate of inactivation, and it cannot make "more active" the already active state of an individual G. Other assumptions, such as the assumption of a single class of single-affinity receptors and assumptions with respect to the potential effects of desensitization and down-regulation, have been discussed previously (1–4).

It should be noted that the model as formulated here requires that the receptor be bound by agonist in order for activation of G to occur. Thus, the encounter coupling would not, without modification, account for spontaneous receptor activity such as has been shown for opioid receptor-mediated stimulation of G in NG108-15 cell membranes (15) or for the constitutive activation of receptors via mutations (16). However, a modified model in which the binding of an agonist to a receptor acts to alter the distribution of the conformational states of the receptor so as to favor such active conformations (10) is easily envisioned. Depending on the rate at which conformational transitions occur, spontaneously or constitutively active receptors would, by such a modified encounter coupling analysis, behave as receptors that are either continuously active (as if continuously bound by agonist) or sporadically active (as if being bound and unbound by agonist). If the conformational transitions were simply an equilibrium between two states (inactive and active) characterized by $K_{eq} = k_1/k_{-1}$, an analysis of spontaneous activation would mathematically be very similar to the foregoing analysis but with both K_{eq} (analogous to $K_d/[A]$) and k_{-1} (equivalent to k_{off}) being unknown variables.

A novel aspect of the encounter coupling model is the asserted relationship of the contributions of both agonist binding frequency and intrinsic agonist-bound receptor efficiency to receptor-mediated cyclase activation. According to the collision coupling model, and based on the expectation and understanding of the proportionality between concentration of agonist-bound receptors and the rate of G activation found in agonist concentration versus cyclase response curves, the relative efficiencies of agonists can be derived solely from the relative extents to which the response curve is separated from the binding curve (the pharmacological shift ratio, $R = K_d/EC_{50}$). Encounter coupling, based on a more detailed consideration of what a collision within a membrane might entail, asserts that the relationship between efficiency and the ratio K_d/EC_{50} can be more complex. Because the relative rate of turnover of occupancy among the receptor population influences the relationship between occupancy and response, the comparison of efficiencies of two agonists is less direct than a comparison of their ratios of K_d/EC_{50}. For instance, as shown in Fig. 7 the difference in K_d/EC_{50} values between two agonists could be attributable, according to the encounter coupling model, solely to differences in binding frequency. This is a somewhat counterintuitive aspect of the system as described by the encounter coupling model. The encounter coupling model asserts that the potency of an efficient agonist can in part depend on a low affinity for receptor, because any degree of receptor occupancy that is rapidly distributed among the entire receptor population will result in greater activation per occupied receptor than would result if occupancy were not so rapidly distributed. Thus, two agonists with identical efficiencies could theoretically have different K_d/EC_{50} ratios solely on the basis of differences in binding frequency, due to the finite lifetime of an encounter. Specifically, consider that a high efficiency, high affinity (long bound lifetime) agonist remains with an encounter 1) longer than is essentially needed to result in G activation and 2) longer than an agonist with a faster rate of dissociation. If the intrinsic efficiencies of these two agonists are equal, then the higher affinity agonist gives a lesser rate of G activation per occupied receptor than does the lower affinity (shorter bound lifetime) agonist.

It is useful to consider, in addition, other factors that influence the extent to which agonist binding frequency alone can contribute to the pharmacological shift ratio, K_d/EC_{50}. It is probable that for some receptor-ligand systems the rate-limiting step in the association of agonist with receptor is the target-finding aspect of the diffusion of the agonist ligand to the receptor at the cell surface (17, 18), rather than the formation of specifically bound complexes. For intact S49 cells, this notion is at least consistent with the appearance that the rate constants for association are in general the same for all receptor ligands, whether agonists or antagonists (3). When association rates are diffusion limited, the specific affinity of ligands for receptors is thus, in general, distinguished by the rate constant for dissociation, such that a high affinity ligand is one that remains bound to a receptor for a longer period of time (18). Thus, there is an offset of the effects of two properties with respect to the ability of the binding frequency of an agonist to increase the net activation rate of G per degree of receptor occupancy for a given intermediate efficiency k_e. On one hand, an agonist with a long receptor-bound state has a greater probability of activation per one encounter for a given k_e. With a lesser affinity, a second agonist with the same efficiency can

increase the number of effective encounters per unit time by decreasing the length of time it spends within one encounter, via a shorter lifetime of the agonist-receptor complex (4), but this is apparently concomitant with a decrease in the affinity of that agonist, relative to the first, and an increase in the agonist concentration required to maintain the same fractional occupancy of receptors (18). For these reasons it is not true that the K_d/EC_{50} ratio is necessarily increased by agonist binding frequency according to the encounter coupling model, but it is clear that there are some combinations of the variables for which differences in the K_d/EC_{50} ratio can, according to the model, be attributed to differences in binding frequency.

A finite encounter duration, as postulated by the encounter coupling model, can also affect the response-blocking capacity of an antagonist. According to the encounter coupling model, the response-blocking activity depends not only on the antagonist concentration and affinity [as predicted by the Cheng-Prusoff relation (eq. 35) and by the collision coupling model] but depends also on its receptor-bound lifetime, relative to the receptor-bound lifetime and binding frequency of the competing agonist. Thus, the encounter coupling model can explain the observation that the effective inhibition constant for an antagonist (K_i) can both differ from its dissociation constant, K_d, and be variable rather than constant, depending on the circumstances in which it is measured (2). That is, the model can account for the effect of antagonists under certain conditions to block agonist activity to an extent that is greater than that predicted by simple competitive occupancy of receptors.

In summary, the encounter coupling model (a revised collision coupling model) can successfully predict agonist concentration versus cyclase response curves by including the rates of agonist association to and dissociation from the receptor, the encounter frequency and duration, and the agonist-bound receptor efficiency as parameters that influence the rate of activation of G. According to the encounter coupling model, the contribution of agonist binding frequency to the rate of G activation is essentially a hidden contributing factor in the agonist concentration versus response curves. The model predicts a rightward shift of the agonist concentration versus response curve for high efficiency agonists when the contribution of binding frequency to the G activation rate is eliminated. The potential effect of an antagonist to restrict the effective rate of the turnover of agonist occupancy among the receptor population, and the potential effect of such a restriction on the rate of G activation in intact cells, can also be successfully modeled by encounter coupling. A quantitative delineation of these aspects of the interactions of agonists, receptors, and G is potentially necessary for a complete understanding of the kinetics of G activation and the regulation of G-mediated responses to agonists in intact cells.

Acknowledgments

We thank Dr. Harvey J. Motulsky for comments and criticism.

Appendix 1. Analytical Solutions to the Encounter Coupling Model Equations for the Probability of Activation of G in One Encounter

Agonist only. The system for the agonist-only case is shown in Fig. 3. In this system the receptor/G pair can be in one of three states, 1) unbound receptor (a) with inactive G ($a + c$), 2) bound receptor with inactive G ($b + c$), and 3) active G with an unspecified receptor state [(a or b) + d]. The solution of interest is that for the single state that includes active G, which is $d(t)$, given by Eq. 9:

$$d(t) = D_0 + D_1e^{\lambda_1 t} + D_2e^{\lambda_2 t} \tag{9}$$

where λ_1 and λ_2 are roots to the quadratic equation:

$$\lambda^2 + d_1\lambda + d_0 = 0 \tag{37}$$

where

$$d_1 = k_{on}[A] + k_{off} + k_e \tag{38}$$

$$d_0 = k_{on}[A]k_e \tag{39}$$

The constants D_i are given by:

$$D_1 = \frac{k_eB_1}{\lambda_1} \tag{40}$$

$$D_2 = \frac{k_eB_2}{\lambda_2} \tag{41}$$

$$D_0 = -(D_1 + D_2) \tag{42}$$

where, for the initial conditions $b = b(0)$, $a(0) = 1 - b(0)$, and $d(0) = 0$:

$$B_1 = \frac{k_{on}[A] - (k_{on}[A] + k_{off} + k_e + \lambda_2)b(0)}{(\lambda_2 - \lambda_1)} \tag{43}$$

$$B_2 = b(0) - B_1 \tag{44}$$

Because the transition to the active state is an irreversible step in the model for the probability of activation in one encounter, the value for $d(\infty)$ must be equal to 1 and, therefore, $D_0 = 1$ ($= -D_1 - D_2$).

Agonist in competition with antagonist. The system for the agonist and antagonist competitive binding case is shown in Fig. 4. In this system the receptor/G pair can be in one of four states, 1) unbound receptor with inactive G ($a + c$), 2) agonist-bound receptor with inactive G ($b + c$), 3) active G with an unspecified state of the receptor [(a or b or h) + d], and 4) antagonist-bound receptor with inactive G ($h + c$). Let

$$k_1 = k_{on}{}^A[A]$$

$$k_2 = k_{off}{}^A$$

$$k_3 = k_{on}{}^B[B]$$

$$k_4 = k_{off}{}^B$$

The solution of interest is for $d(t)$, given by eq. 34:

$$d(t) = D_0 + D_1e^{\lambda_1 t} + D_2e^{\lambda_2 t} + D_3e^{\lambda_3 t} \tag{34}$$

where λ_1, λ_2, and λ_3 are roots to the cubic equation:

$$\lambda^3 + d_2\lambda^2 + d_1\lambda + d_0 = 0 \tag{45}$$

where

$$d_2 = k_1 + k_2 + k_3 + k_4 + k_e \tag{46}$$

$$d_1 = k_1(k_2 + k_3 + k_e) + k_2(k_4 + k_e) + k_3k_e \tag{47}$$

$$d_0 = k_1k_3k_e \tag{48}$$

For the initial condition $a = a(0)$ and $d = d(0) = 0$:

$$D_1 = \frac{k_e B_1}{\lambda_1} \quad (49)$$

$$D_2 = \frac{k_e B_2}{\lambda_2} \quad (50)$$

$$D_3 = \frac{k_e B_3}{\lambda_3} \quad (51)$$

$$D_0 = -(D_1 + D_2 + D_3) \quad (52)$$

where

$$B_3 = \frac{(G - D_1\lambda_1^2 - D_2 - D_2\lambda_2^2)}{\lambda_3^2} \quad (53)$$

$$B_2 = \frac{(F - G - D_1(\lambda_1\lambda_3 - \lambda_1^2))}{(\lambda_2\lambda_3 - \lambda_2^2)} \quad (54)$$

$$B_1 = \frac{(b(0)\lambda_3^2 - G)(\lambda_2\lambda_3 - \lambda_2^2) - (F\lambda_3 - G)(\lambda_3^2 - \lambda_2^2)}{(\lambda_3^2 - \lambda_1^2)(\lambda_2\lambda_3 - \lambda_2^2) - (\lambda_3^2 - \lambda_2^2)(\lambda_1\lambda_3 - \lambda_1^2)} \quad (55)$$

where

$$F = \left.\frac{db}{dt}\right|_{t=0} = k_1 a(0) - (k_4 + k_e)b(0) \quad (56)$$

$$G = \left.\frac{d^2b}{dt^2}\right|_{t=0} = k_1 \left.\frac{da}{dt}\right|_{t=0} - (k_4 + k_e)\left.\frac{db}{dt}\right|_{t=0} \quad (57)$$

where

$$\left.\frac{da}{dt}\right|_{t=0} = k_4 h(0) - (k_1 + k_3)a(0) + k_2 b(0) \quad (58)$$

and where $a(0)$, $b(0)$, and $h(0)$ are the initial conditions for a, b, and h, given

$$h(0) + a(0) + b(0) = 1 \quad (59)$$

$$d(0) = 0 \quad (60)$$

As for the agonist-only case, the value for $d(\infty)$ must equal 1 and, therefore, $D_0 = 1(= -D_1 - D_2 - D_3)$.

Appendix 2. Derivation of $k_a = P\omega$

If P is the probability of activation in one encounter, then $(1 - P)$ is the probability of no activation in one encounter, P_0. The probability of no activation in n encounters, $P_0(n)$, is given by:

$$P_0(n) = (1 - P)^n \quad (61)$$

The probability that exactly n encounters occur in time t, $P(n,t)$, is given by a single term in a Poisson distribution:

$$P(n, t) = e^{-\omega t}\frac{(\omega t)^n}{n!} \quad (62)$$

where ω is the mean frequency with which encounters occur. Therefore, the probability of no activation in time t, $P_0(t)$, is given by the summation of the probabilities for all possible numbers of encounters:

$$P_0(t) = \sum_{n=0}^{n=\infty} P_0(n)P(n, t) \quad (63)$$

or

$$P_0(t) = \sum_{n=0}^{n=\infty} (1 - P)^n e^{-\omega t}\frac{(\omega t)^n}{n!} \quad (64)$$

This can be rewritten:

$$P_0(t) = e^{-\omega t}\sum_{n=0}^{n=\infty}\frac{[(1 - P)\omega t]^n}{n!} \quad (65)$$

$$P_0(t) = e^{-\omega t}e^{(1-P)\omega t} \quad (66)$$

$$P_0(t) = e^{-\omega t}e^{\omega t}e^{-P\omega t} \quad (67)$$

$$P_0(t) = e^{-P\omega t} \quad (68)$$

The probability of activation $P_a(t)$ in time t is thus:

$$P_a(t) = 1 - P_0(t) \quad (69)$$

$$P_a(t) = 1 - e^{-P\omega t} \quad (70)$$

This expression for $P_a(t)$ can be equated to the overall probability of activation in time t, $P_a(t)$, that is characterized by a rate constant k_a:

$$P_a(t) = 1 - e^{-k_a t} \quad (71)$$

from which

$$e^{-k_a t} = e^{-P\omega t} \quad (72)$$

and

$$k_a = P\omega \quad (73)$$

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Send reprint requests to: Douglas Stickle, Department of Biological Chemistry, Pennsylvania State University College of Medicine, Hershey, PA 17033.