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THE ENCOUNTER COUPLING MODEL FOR β-ADRENERGIC RECEPTOR/GTP-BINDING PROTEIN INTERACTION IN THE S49 CELL

CALCULATION OF THE ENCOUNTER FREQUENCY

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Abstract—Experiments measuring epinephrine stimulation of the S49 cell have demonstrated that the rate of adenylate cyclase activation is partly dependent on the rate of turnover of epinephrine occupancy with respect to individual receptors. Specifically, it has been shown that a low occupancy of the full receptor population by epinephrine promotes a rate of adenylate cyclase activation significantly greater than that for a low number of receptors completely occupied by epinephrine with which the concentration of bound receptors is the same. This finding indicated that the interaction of individual receptors with GTP-binding protein (G) occurs on a time scale which is greater than the mean lifetime of the epinephrine–receptor complex; during this period of interaction (an "encounter"), a receptor can change its occupancy state in the presence of a high binding frequency agonist such as epinephrine. Here we present a general analysis, in an extension of the Collision Coupling Model of Tolkovsky and Levitzki (*Biochemistry* **17**: 3795–3810, 1978), of the consequences of encounters (rather than pure collisions) for the relationships of receptor occupancy, receptor–agonist complex lifetime, and receptor–agonist efficiency to G/adenylate cyclase activation. Using this "encounter coupling" model of receptor/G interaction, it is demonstrated from a theoretical standpoint that the net rate of G activation can depend in part on the agonist binding frequency. The predicted dependence is consistent with the data on which the model is based, in which high binding frequency increases the activation rate. A special case of the "encounter coupling model" allows calculation of the frequency of encounters by an analysis of a previous experiment using epinephrine in which the rate of adenylate cyclase activation was measured in response to a small number of fully occupied, highly efficient receptors. Using those results and the model developed here, the encounter frequency was found to be on the order of 100/min in the intact S49 cell. This calculation relied on knowledge of the rate of inactivation of G/adenylate cyclase in intact cells. A method for the measurement of the adenylate cyclase inactivation rate is presented. Using this method, the adenylate cyclase inactivation rate constant was found to be between 0.8 and 3.0/min.

β-Adrenergic receptor agonists stimulate the production of cAMP via the activation of GTP-binding proteins (G) coupled to adenylate cyclase [1]. In intact cells, the activity of cyclase (the rate of cAMP generation) in response to a given concentration of agonist is not, however, proportional to the occupancy of receptors by agonist. In fact, the fractional activity is invariably greater than the fractional receptor occupancy; for some agonists, near-maximal cyclase activity occurs when the fractional receptor occupancy by agonist is quite low. This relationship may be viewed as a "separation" between the response and receptor occupancy curves as a function of agonist concentration. For a given agonist, the separation is usefully characterized by the ratio of the concentration required for 50% receptor occupancy (K_d) to the concentration required for 50% activity (EC_{50}). This ratio, also known as the Pharmacological Shift Ratio (PSR = K_d/EC_{50}) [2], can be as high as 200 in intact S49 murine lymphoma cells, as in the case of epinephrine (EC_{50} = 10 nM, K_d = 2 μM), i.e. the concentration of epinephrine that stimulates half-maximal cyclase activity is 200-fold less than the concentration of epinephrine which results in half-maximal receptor occupancy [3].

The relationship of the occupancy and activity curves can be explained by the Collision Coupling Model of Tolkovsky and Levitzki [4], in which (1) any one G protein is accessed by numerous receptors per unit time (via collisions due to free diffusion of the two species), such that the *rate* of activation of G (and of cyclase) is proportional to the concentration of agonist-bound receptors, and (2) the inactivation of cyclase occurs by a process that is independent of receptor activity. The collision coupling model incorporated the model of Cassel and co-workers for the GTP–GDP exchange cycle of G [5–7], along with the assumption that inactivation of cyclase was kinetically equivalent to GTP hydrolysis by G (i.e. that cyclase was active in proportion to the presence of active G) as was demonstrated in turkey erythrocytes [8].

According to collision coupling, the fractional

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cyclase activity f is thus a function of two rate constants: the rate constant for activation, k_a, and the rate constant for inactivation, k_i: $f = k_a/(k_a + k_i)$ [9]. The rate constant k_a for cyclase activation is proportional to the frequency of collisions between receptors and G, to receptor occupancy (the probability that a collision involves a receptor that is in the bound state), and to an agonist-dependent factor reflecting the efficiency of each collision (the probability that a collision is successful at activation). The collision coupling model not only predicts an essential separation between the occupancy and response curves as a function of agonist concentration, but also describes the relationship of the degree of separation (the K_d/EC_{50} ratio, or PSR) to the maximum fractional cyclase activation, f, which can be obtained by a given agonist. According to the model, the differences in PSRs among different agonists are attributable solely to differences in the efficiency factor [9].

An implicit assumption of the collision coupling model is that collisions between receptor and G are isolated, independent events of zero duration. Specifically, if the rate of activation via collisions is strictly proportional to the concentration of agonist-bound receptors, then a collision must occur with a receptor that is strictly in one of two states, either bound to agonist or unbound. A later model developed by Swillens [10] considered the consequences of an alternative assumption for which collision coupling is a special case. In consideration of the fact that these interactions occur within the densely packed framework of the lipid bilayer membrane, it was reasoned that the interaction of any one receptor/G pair could occur in isolated episodes (an encounter) that consisted of a finite series of collisions within a finite time. Such a model detailed the *a priori* possibility that the relative rates of two additional processes could affect the rate of adenylate cyclase activation: (1) the rate at which receptors and G, once engaged in an encounter, leave the encounter (a rate related to the mean duration of the period in which receptors and G remain in interactive proximity), and (2) the rate of dissociation of agonist from the receptor. The intrinsic agonist efficiency was defined as a rate constant reflecting the probability per unit time that the receptor–agonist complex can catalyze G activation during an encounter. It follows from Swillens' model that under some conditions the simple collision coupling model may lead to incorrect predictions. For instance, in the collision coupling model, where the rate of activation is proportional to the concentration of agonist-bound receptors [RA], the extent of adenylate cyclase activation should be the same for a given [RA] whether it is obtained as the result of a large concentration of receptors with a low occupancy by agonist (i.e. with each receptor occupied by agonist a small fraction of the time) or obtained by a smaller receptor concentration with a compensatingly higher agonist occupancy. According to Swillens' model, the two cases by which a given [RA] is obtained could theoretically yield different rates and extent of G activation, under the combination of conditions in which frequency of the episodes of interactions between G and receptor is low, the activating efficiency of the agonist is high, and the agonist–receptor complex has a short lifetime (corresponding to high agonist binding frequency, or rapid dissociation of agonist from the receptor) relative to the duration of the interaction between R and G.

In an earlier paper we demonstrated that such a combination of conditions appears to exist for epinephrine activation of adenylate cyclase in the S49 cell [3]. The antagonist propranolol was used in a protocol designed so that most of the receptors were blocked by propranolol most of the time, whereas the remainder of the receptors were occupied almost continuously by epinephrine. This experiment approached a condition in which the rate of adenylate cyclase activation was limited by the diffusion rate of a low number of fully occupied receptors, such that any potential contribution of binding frequency was largely eliminated by severely restricting the rate of sharing (or "switching") of occupancy among the entire receptor population. The rate of cyclase activation for this condition was compared to that for the point on the control concentration versus response curve, in the absence of antagonist, at which the same concentration of agonist-bound receptors was achieved by low occupancy of the full number of receptors. The rate of activation for the first case (the receptor diffusion-limited case) was significantly less than for the control case (which allows both receptor diffusion plus agonist binding and unbinding), demonstrating that the rate of turnover of agonist occupancy with respect to individual β-adrenergic receptors contributes significantly to the adenylate cyclase activation rate by epinephrine in the S49 cell in the region of the concentration/response curve near the EC_{50} [3]. Thus, there are circumstances for which the collision coupling model is insufficiently detailed with respect to the exact nature of collisions, since the rate of cyclase activation is not under all circumstances proportional to the concentration of agonist-bound receptors.

This observable effect on cyclase activation upon restriction of the switching of epinephrine occupancy among receptors can be explained by the concept of "encounters" and the combination of factors entailed with periodic episodes of collisions between R and G as described above. First, the encounter apparently involves some finite time; in terms of pure collisions, this would mean that individual receptors and G collide with each other a number of times in sequence if they collide at all. Second, epinephrine is bound for a relatively short time to any one receptor compared to the mean duration of an encounter. That is, a receptor can apparently change state with respect to epinephrine occupancy within the time scale on which encounters take place, such that any epinephrine-bound receptor within an encounter does not necessarily arrive at that encounter with epinephrine already bound, and it will not necessarily leave the encounter while epinephrine is still bound. Third, epinephrine-bound receptors are apparently very efficient (per collision) at activating G; that is, considering the relationship between overall receptor occupancy and response (that only low occupancy is needed to promote near-maximal response), the

occupancy attributable to any one epinephrine-bound receptor apparently does not "waste" many collisions in a single place with a single G which it has already activated. Instead, that occupancy is rapidly redistributed and shared amoung the entire receptor population, thereby increasing the net number per unit time of *different* encounters with *inactive* G over the number possible when the same concentration of agonist-bound receptors involves only a small fraction of the full complement of receptors.

In this paper we consider a number of aspects of this problem in more quantitative detail and in terms of an encounter coupling model which takes these factors into account. First, we examine the potential influence of agonist binding frequency on the extent of G activation per extent of receptor occupancy within the context of such a model. Using calculations for two extreme cases of the relationship of the receptor–agonist complex lifetime to the encounter duration, it is demonstrated from a theoretical standpoint that, given such a model, agonist binding frequency can be a significant factor in the relationship between occupancy and response for an efficient agonist.

Second, we analyze a previously published experiment with respect to the encounter coupling model, as a case analogous to one of the above extreme cases of the relationship of the receptor–agonist complex lifetime to the encounter duration. It is shown that the data from that experiment provide a means to calculate the encounter frequency between receptor and G in the S49 cell (a probability per unit time that any one G interacts with a receptor). The calculation of encounter frequency relies on knowledge of the inactivation rate constant (k_i) for activated adenylate cyclase in intact cells. A portion of this work, therefore, involves measurement of k_i, which was found to be on the order of 1.5/min for the S49 cell. Using this value for k_i, the encounter frequency between receptors and G is estimated to be on the order of 100/min.

EXPERIMENTAL PROCEDURES

Measurement of the adenylate cyclase inactivation rate, k_i

The rate constant for adenylate cyclase inactivation can be derived from an analysis of the time course of cAMP generation, following procedures developed by Cassel and coworkers [6, 7]. First, assume that in the presence of a fixed concentration of agonist (and some steady-state value for the concentration of agonist-bound receptors), the rate of activation of cyclase from E (the inactive enzyme state) to E* (the active state) is first order with respect to the concentration of inactive enzyme E, and that its inactivation rate is first order with respect to the concentration of active enzyme E*. Then

$$\frac{d[E^*]}{dt} = k_a[E] - k_i[E^*] \quad (1)$$

where k_a is the activation rate constant and k_i is the inactivation rate constant. With the initial condition $E^*(0) = 0$ (prior to agonist addition), the concentration of E* as a function of time is given by

$$[E^*](t) = [E]_T \frac{k_a}{(k_a + k_i)}(1 - e^{-(k_a + k_i)t}) \quad (2)$$

where $[E]_T$ is the total enzyme concentration. Second, assume that the rate of cAMP generation is proportional to E*. At sufficiently early times, the rate of accumulation of cAMP in intact cells is largely attributable to its rate of generation [11]. Thus, ignoring (for the moment) the negative contribution of cAMP degradation to the mass balance for cAMP, the rate of cAMP accumulation is given by:

$$\frac{d[\text{cAMP}]}{dt} = k_g[E^*]$$

Then

$$[\text{cAMP}](t) = \int_0^t k_g[E^*]dt \quad (3)$$

Combining Equations (2) and (3) and integrating:

$$[\text{cAMP}](t) = k_g k_a \frac{[E]_T}{(k_a + k_i)}\left(t + \frac{(e^{-(k_a - k_i)t} - 1)}{(k_a + k_i)}\right) \quad (4)$$

for the initial condition cAMP(0) = 0. According to these assumptions, [E*] approaches a steady state, and [cAMP](t) becomes linear:

$$[\text{cAMP}](t) = k_g k_a \frac{[E]_T}{(k_a + k_i)}\left(t - \frac{1}{(k_a + k_i)}\right) \quad (5)$$

which has the t-axis intercept (t_{int})

$$t_{int} = \frac{1}{(k_a + k_i)} \quad (6)$$

The ratio of the rate constants k_i and k_a can be determined from the concentration of agonist used and the EC_{50} for that agonist: the steady-state fractional cyclase activation f ($=[E^*]/[E]_T$) at agonist concentration [A] is given empirically by:

$$f = \frac{[A]}{[A] + EC_{50}} \quad (7)$$

From Equation (1), for $d[E^*]/dt = 0$, the steady-state fractional cyclase activation f ($=[E^*]/[E]_T$) at agonist concentration [A] is also given by:

$$f = \frac{k_a}{k_a + k_i} \quad (8)$$

From Equation (8) the ratio of k_i to k_a is thus given by

$$\frac{k_i}{k_a} = \frac{1 - f}{f}$$

and

$$k_a = \frac{f k_i}{(1 - f)} \quad (9)$$

Equations (6) and (9) can be combined to give the relationship between k_i and t_{int}:

$$k_i = \frac{1 - f}{t_{int}} \quad (10)$$

k_i can therefore be determined using Equation (10) from t_{int}, given [A] and the EC_{50}, by substituting Equation (7) for f and rearranging:

$$k_i = \frac{1}{\left(\frac{[A]}{EC_{50}} + 1\right) t_{int}} \quad (11)$$

For example, using a concentration of agonist [A] equal to its EC_{50}, then k_i from Equation (11) is given by:

$$k_i = \frac{1}{2t_{int}}$$

Experiments were conducted to determine k_i using epinephrine (EC_{50} = 10 nM), isoproterenol (EC_{50} = 2 nM) and zinterol (EC_{50} = 2 nM). Measurement of cAMP accumulation (percent conversion of [^{3}H]ATP to [^{3}H]cAMP in cells prelabeled with [^{3}H]-adenine) followed previously described procedures [3, 12].

THEORY AND MATHEMATICAL MODEL FOR RECEPTOR/G PROTEIN ENCOUNTER COUPLING

Here we present derivations for the relationship between encounter frequency, encounter duration, agonist occupancy, agonist-dependent receptor efficiency and the extent of G protein/adenylate cyclase activation for two different cases of the mean lifetime of the receptor–agonist complex relative to the mean lifetime of an encounter. First is the case in which the mean duration of the agonist-bound state is short compared to the encounter duration. Second is the case in which the mean duration of the agonist-bound state is long compared to the duration of an encounter. For each, we first derive an expression for the probability of G/cyclase activation in a single encounter (assuming the kinetic equivalence of G activation and cyclase activation [8, 10]). From this we derive an expression for the overall rate of activation, and the resultant fractional cyclase activation, given the rate at which such encounters occur. The second case (long mean bound lifetime, effectively equivalent to high agonist occupancy during a single encounter) is shown to correspond to conditions in an experiment previously reported [3] (high occupancy by agonist of a small number of receptors, with which the receptor complex has a high efficiency at cyclase activation), from which the encounter frequency can be calculated.

Probability of cyclase activation in one encounter

Consider a case in which an encounter between receptor and G takes place with a fixed, finite time of duration τ. There are two cases to be considered with respect to the occupancy of the receptor during an encounter (Fig. 1). The first is where the mean lifetime of the receptor–agonist complex (t_m) is short compared to τ. The second is where the mean lifetime of the receptor–agonist complex t_m is long compared to τ.

For both cases we will assume that the activation of G is a first-order process operating during the time when agonist is present on the receptor and

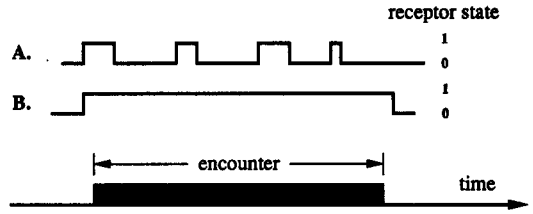

Fig. 1. Two cases for the relationship of receptor–agonist complex lifetime and receptor occupancy by agonist to the encounter time: (A) short lifetime compared to encounter time; (B) long lifetime compared to encounter time. The receptor state is represented as a binary variable: 0 = unbound, 1 = bound to agonist. Note that in the illustration the overall receptor occupancy by agonist for case B is indeterminate, since only one cycle of agonist binding is shown, whereas for case A the overall receptor occupancy by agonist (which is an arbitrary aspect of the illustration) could be estimated from the fraction of time spent in the bound state.

when the receptor and G are involved in an encounter, and calculate the probability of activation in a single encounter for these two conditions. The rate constant for this process is an intrinsic agonist-dependant rate constant, k, for the efficiency of the agonist-occupied receptor, that is presumed to be a direct reflection of the ability of the specific conformational state of the receptor, engendered by occupancy by a particular agonist, to activate G.

Short lifetime of the agonist–receptor complex ($t_m \ll \tau$). If the mean lifetime of the receptor–agonist complex is short compared to the encounter time, then agonist may be on and off the receptor many times during an encounter. For the limiting case where the receptor–agonist complex lifetime is vanishingly small, all encounters will be equivalent and in all cases agonist will be present for a fraction θ of the time during the encounter, where θ is the receptor occupancy (i.e. the probability that any one receptor is bound to agonist at any given instant, or, equivalently, the fraction of time that any one receptor is bound to agonist). If the rate constant for activation is k, then the probability of G being activated during a random encounter of duration τ is given by

$$P_{a,1} = 1 - e^{-\theta k \tau}$$

Long lifetime of the agonist–receptor complex ($t_m \gg \tau$). When the lifetime of the receptor–agonist complex is long compared to the encounter time, the probabilities of activation are described by different considerations. In contrast to the previous case, any encounter which begins with agonist present is likely to end with the agonist still present. The probability $P_{a,1^*}$ of G being activated during a single encounter with agonist present is therefore given by

$$P_{a,1^*} = 1 - e^{-k\tau}$$

The probability P_a of G being activated during a random encounter is therefore given by the probability of activation during one encounter with agonist present ($=1 - e^{-k\tau}$) multiplied by the probability that agonist is present (θ):

$$P_{a,1} = \theta\,(P_{a,1^*}) = \theta\,(1 - e^{-k\tau})$$

where θ is the receptor occupancy (again, the probability that any one receptor is bound to agonist or, equivalently, the fraction of receptors which are bound to agonist).

Probability of cyclase activation given n *encounters per time* t

Given the probability of activation in a single encounter, the overall activation of G can be calculated for each case as a function of the frequency with which encounters occur as shown below.

Short lifetime of the receptor–agonist complex ($t_m \ll \tau$). A receptor may be bound and unbound a number of times during an encounter when the agonist–receptor complex has a comparatively short lifetime (Fig. 1). Again let the occupancy of the receptor = θ (= the fraction of time bound, ranging from 0 to 1) and let there be a fixed probability per unit time that activation of G protein takes place when a receptor is bound and within an encounter. Then, from the derivation given above, the probability of activation during one encounter $P_{a,1}$ is given by

$$P_{a,1} = 1 - e^{-\theta k \tau}$$

The probability of *no* activation during one encounter $P_{0,1}$ is thus given by

$$P_{0,1} = e^{-\theta k \tau}$$

If n encounters take place, then the probability of no activation during n encounters, $P_{0,n}$ is given by

$$P_{0,n} = (e^{-\theta k \tau})^n = e^{-n\theta k \tau}$$

Therefore, the probability of activation during n encounters $P_{a,n}$ is given by

$$P_{a,n} = 1 - e^{-\theta k \tau}$$

If n encounters occur during time t, then $P_{a,n}$ is related to the overall rate constant for G activation (k_a) by

$$P_{a,n} = 1 - e^{-k_a t}$$

Therefore

$$1 - e^{-k_a t} = 1 - e^{-n\theta k \tau}$$

$$k_a t = n\theta k \tau$$

and thus

$$\frac{n}{t} = \frac{k_a}{\theta k \tau}$$

where the ratio n/t, or number of encounters per time, is the encounter frequency, k_c:

$$k_c = \frac{k_a}{\theta k \tau}$$

Assuming that the activation of G and cyclase are kinetically equivalent [8], then the quantity k_a is calculated from the overall fractional cyclase activation f, according to the Cassel and Selinger model [7] of cyclase activation/inactivation:

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

f is derived from measurements of cAMP accumulation as described earlier [3]; 1 min cAMP accumulation is proportional to the maximum rate of cAMP generation (the cyclase activity) for a particular set of conditions; when divided by the maximal possible 1 min cAMP accumulation, the cAMP accumulation under those conditions can be related to the fractional cyclase activity, f [12]. Given f, then

$$k_a = \frac{k_i f}{(1 - f)}$$

where k_i is the cyclase inactivation rate constant. Thus

$$k_c = \frac{k_i f}{(1 - f)\,\theta k \tau}$$

Long lifetime of the receptor–agonist complex ($t_m \gg \tau$). When the lifetime of the receptor–agonist complex is long compared to the encounter time, the derivation of encounter frequency from activation rate is more complex. A special case for which the derivation is simple is where the probability of activation during an encounter is high. If the probability of activation during an encounter P_a is equal to 1, then the activation rate k_a is simply equal to the encounter rate between agonist-bound receptors and cyclase. Thus (activation rate = encounter rate × probability of encounter with bound receptor):

$$k_a = \theta\,(n/t)$$

$$k_c = (n/t) = k_a/\theta$$

$$k_c = \frac{k_i f}{(1 - f)\theta}$$

For reasons discussed in detail below, this case is used for the calculation of k_c.

RESULTS

Adenylate cyclase inactivation rate, k_i

The results of experiments designed to measure the cyclase inactivation rate constant using three different agonists are shown in Fig. 2. Using epinephrine (10 nM, EC_{50} = 10 nM) at 25°, a line drawn through the straight portion of the data gives a t-axis intercept between 19 and 24 sec (Fig. 2A). Using the equations derived above, $t_{int} = 1/(2k_i)$ when [A] = EC_{50}. The t-axis intercept for this experiment thus corresponds to k_i = 1.3 to 1.6/min at 25°.

The value obtained for k_i by this method should be independent of the stimulating agonist. The results of an experiment using isoproterenol (5 nM, EC_{50} = 2 nM) at 25° are shown in Fig. 2B. A line drawn through the straight portion of the data gives a t-axis intercept between 15 and 17 sec. In this case [agonist] = 2.5EC_{50}, such that $t_{int} = 1/(3.5k_i)$. The t_{int} thus corresponds to k_i = 1.0 to 1.1/min at 25°. The results of an experiment using zinterol, which is a relatively poor agonist in this system, are shown in Fig. 2C for 37° (10 nM, EC_{50} = 2 nM). A line drawn through the straight portion of the data gives a t-axis intercept of between 11 and 12 sec. For [zinterol] = 5EC_{50}, $t_{int} = 1/(6k_i)$; for this experiment, then, k_i = 0.8 to 0.9/min at 37°.

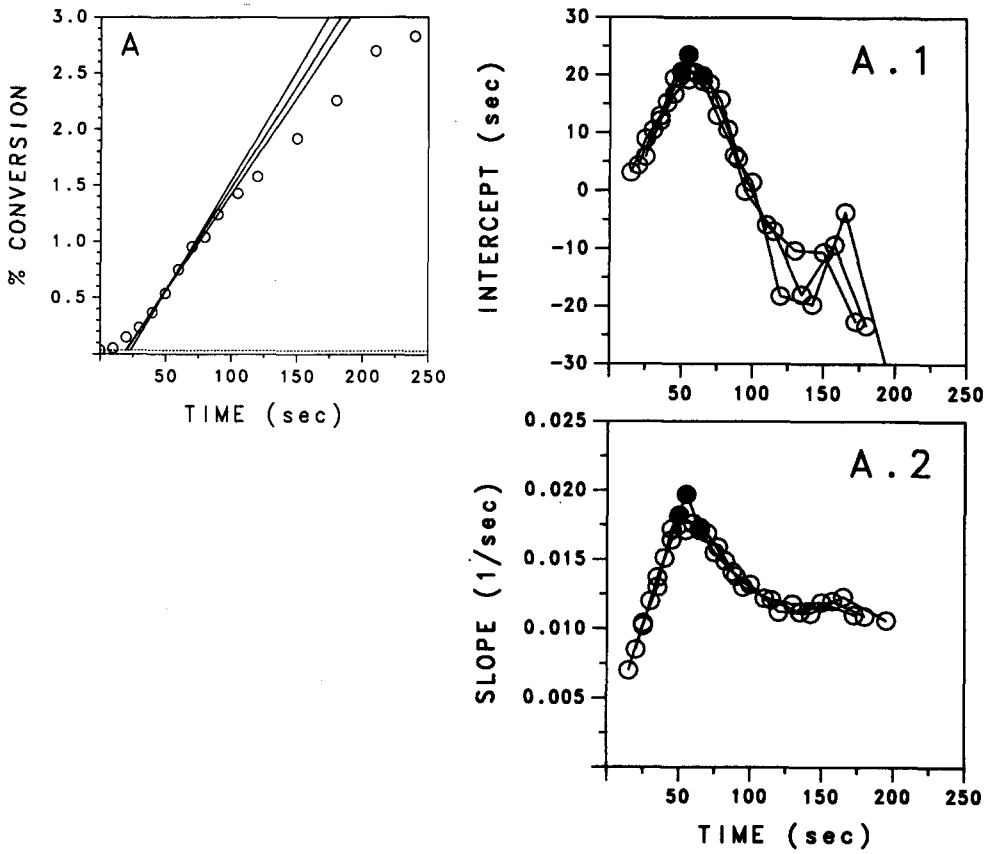

Fig. 2. Representative time courses of cAMP accumulation for measurement of k_i. (A) 10 nM epinephrine (EC_{50} = 10 nM), 25°. (B) 5 nM isoproternol (EC_{50} = 2 nM), 25°. (C) 10 nM zinterol (EC_{50} = 2 nM), 27°. The lines drawn in each case are the lines of the maximum slopes and t-intercepts obtained by linear regression of contiguous sets of 3, 4 and 5 data points. The associated figures show the calculated t-intercepts (A.1, B.1 and C.1) and slopes (A.2, B.2, and C.2), with the maximum values for sets of 3, 4 and 5 points shown as filled circles (●).

The determinations for k_i from the time course of cAMP accumulation in intact cells as described above are potentially complicated by the effects of ongoing desensitization and of cAMP hydrolysis that are not explicitly accounted for in Equation (11). These influences result in cAMP accumulations which do not attain the linearity seen in the cell-free adenylate cyclase assays. In cell-free assays, the slope of the accumulation curve increases to an asymptote at which time the rate of cAMP synthesis is maximal. With intact cells, the accumulation curve is initially made concave upwards due to the lag time in achievement of a transient steady state, and it is later made convex upwards due to effects of both cAMP hydrolysis and desensitization. Both of these effects also tend to reduce the maximum slope obtainable in such an experiment. Taking the maximum slope, therefore, represents the best approach for the estimate of k_i in intact cells. The lines drawn through the data in Fig. 2 were derived, therefore, from an analysis of the data to determine the maximum rate of cAMP accumulation obtained in each experiment. The analysis consisted of a series of linear regression calculations for sets of contiguous data points, using set sizes of 3, 4 and 5 data points. The values and ranges for t_{int} were determined from the intersection of those lines with the baseline (time = 0) percent conversion. The results of the calculations of slopes and intercepts are shown in associated panels A.1 and A.2, B.1 and B.2, and C.1 and C.2 of Fig. 2A, 2B and 2C. From this analysis it can be seen that both the maximum slope and the maximum baseline intercepts occur simultaneously. The maximum correlation coefficients for the calculated lines also occur at these times (data not shown).

In intact S49 cells the fractional turnover constant for cAMP is in the region of 0.1 min^{-1} [11]. Thus, the effects of hydrolysis on cAMP accumulation are small at relatively early times after stimulation. For instance, the cAMP accumulation in 1 min is a good single-point measure of the maximum rate of cAMP obtained in each of these experiments. Since the potential effects of desensitization and hydrolysis are to reduce both the maximum slope and the maximum t_{int} in such experiments, the values

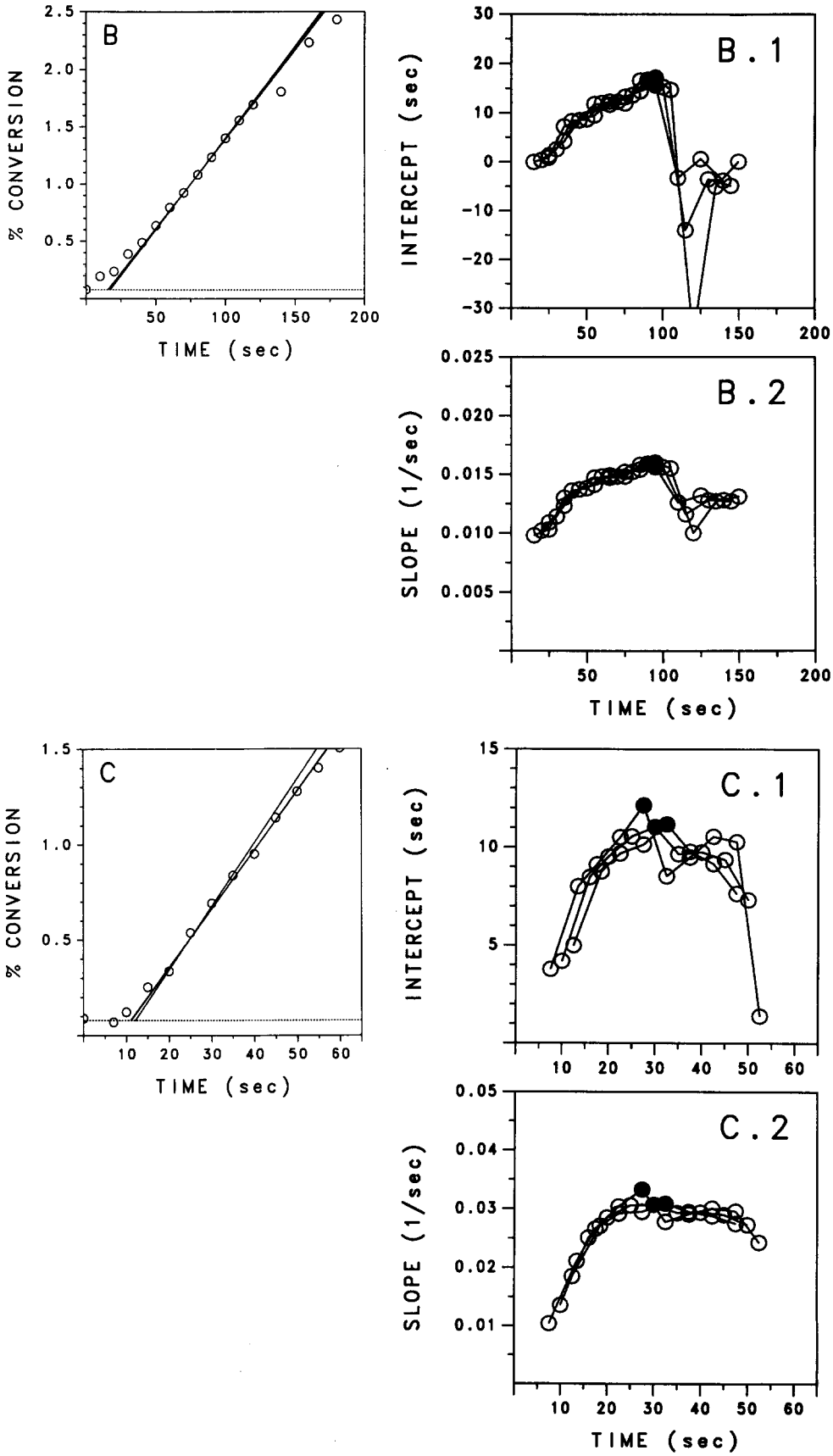

Fig. 2. Continued.

obtained for t_{int} are minimum values, and therefore the values calculated for k_i are maximum values. Experiments at 37° using epinephrine and isoproterenol exhibited slightly more pronounced effects of cAMP hydrolysis and desensitization than at 25°. Numerous experiments using epinephrine and isoproterenol at 37° nonetheless gave relatively similar and expectedly greater values for k_i ranging from $k_i = 2.0$/min to $k_i = 3.0$/min (data not shown). Thus, all the k_i values obtained using three different agonists at two temperatures fell within a reasonably small range ($k_i = 0.8$ to 3.0/min).

Calculation of encounter frequency, k_c

The basis for the calculation of encounter frequency k_c is an experiment using an epinephrine/propranolol experiment reported previously [3]. This experiment was designed as a special case for receptor occupancy by epinephrine. Normally epinephrine occupancy is rapidly distributed among the entire receptor population on a short time scale, such that average occupancy θ is equivalent to the fraction of time a receptor is bound. In the experiment, the average occupancy θ was increasingly isolated to a smaller fraction of the receptor population using increasing concentrations of propranolol, a condition which can be achieved because of the long (compared to epinephrine) half-life of the propranolol–receptor complex. The results of the experiment are reproduced in Fig. 3. Conditions in this experiment approached one in which the fraction θ of the receptors were kept occupied by epinephrine for long periods of time, whereas the remainder of the receptors were blocked by the antagonist propranolol. This is most true for the rightmost point in Fig. 3 wherein the concentrations of both epinephrine and propranolol were the highest used (10 μM epinephrine, 215 nM propranolol). Thus, for every encounter between receptor and cyclase that could possibly lead to activation (i.e. those for which the receptor was bound by epinephrine for any portion of the encounter), the receptor was most probably occupied during the entire encounter. For that point in Fig. 3 approximately 30 of 2000 receptors were occupied almost continuously. This gives an average occupancy θ of 30/2000, or 0.015. Were the isolation of agonist occupancy to a fixed number of receptors absolute, then for every epinephrine-bound receptor encountered by a G it will on average have encountered 2000/30 ($=1/\theta$) times that number of receptors total.

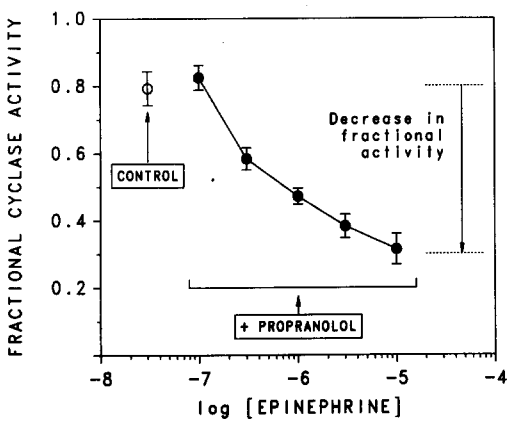

Fig. 3. Effect on adenylate cyclase activity in intact S49 cells of reductions in the number of available receptors in the presence of a fixed and constant concentration of epinephrine-bound receptors. The effective number of available receptors was in each case reduced (compared to control) by the addition of propranolol, while a fixed concentration of epinephrine-bound receptors (equal to control) was in each case maintained by a compensating increase in the amount of epinephrine. Abscissa: final epinephrine concentration (M). Although the actual numbers are not shown for propranolol, both the propranolol and epinephrine concentrations are increasing from left to right. Ordinate: adenylate cyclase activity [1 min cAMP accumulation (% conversion) expressed as a fraction of maximum; mean ± SEM (N = 3)]. These data were originally presented in Ref. 3.

Moreover, the efficiency of activation for this circumstance is very high, i.e. there is a very high probability that the encounter will lead to G/adenylate cyclase activation when the encounter involves a receptor which is occupied continuously by epinephrine during the entire encounter. The cyclase activation rate constant k_a has thus been measured for the special case wherein it approximates a direct measure of the encounter rate between G and agonist-bound receptors, i.e. the activating frequency (frequency of successful encounters) is equal to the frequency of encounters with agonist-bound receptors. As described above, the overall (total) number of encounters per unit time (i.e. the encounter rate for encounters with both bound and unbound receptors) is then given by the k_a measured under this condition, divided by θ:

$$k_c = k_a/\theta$$

The fractional cyclase activity f was measured for this case to be $f = 0.3$ at 37°. The activation rate constant k_a is calculated using Equation (9): $k_a = k_i f/(1-f)$. For $k_i = 0.8$/min, $k_a = 0.34$/min. For $k_i = 3.0$/min, $k_a = 1.3$/min. An initial estimate for the total encounter frequency falls within the range calculated using these boundary values for k_i:

$$k_c > 0.34\text{/min}/\theta = 0.34\text{/min}/0.015 = 23\text{/min}$$
$$k_c < 1.30\text{/min}/\theta = 1.30\text{/min}/0.015 = 87\text{/min}$$

These initial estimates for k_c are made on the basis of specific values for both θ and f and a range of k_i values; the dependence of the calculated k_c on θ and f for a range of values for θ and f, near $\theta = 0.015$ and $f = 0.3$, is shown in Fig. 4. In ranges of values for $\theta = 0.01$ to 0.02 and for $f = 0.2$ to 0.4, the initial calculated value for k_c can vary by a factor that is on the order of 2. An added consideration in the calculation of k_c is that the isolation of receptor occupancy, in the experiments from which k_c is derived, only approached the ideal case of a fixed number of receptors, and the actual number of receptors involved with agonist over the 1-min time course of the experiments is greater than exactly 1.5% of the total number of receptors [9]. Thus, the number of receptors interacting with any one G must be somewhat greater than k_a/θ, and both of the limits on k_c as calculated above are minimum values.

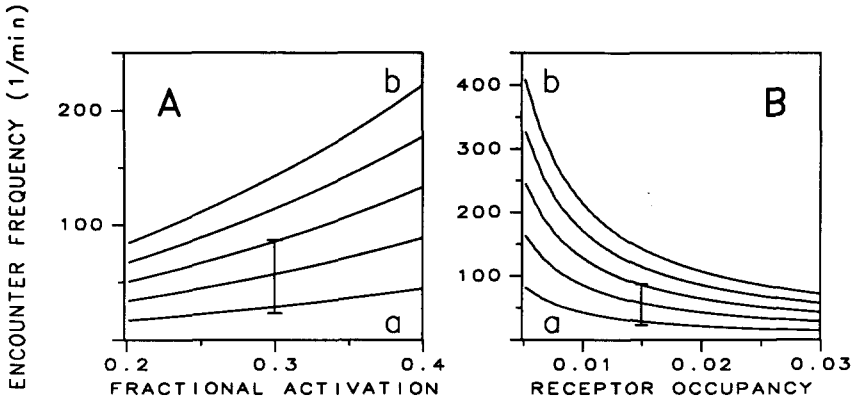

Fig. 4. Dependence of the calculated encounter frequency k_c on the fractional activation f (A) and the receptor occupancy θ (B), with the cyclase inactivation rate constant k_i as a parameter. The rate constant k_i ranges from 1/min (a) to 5/min (b). The initial estimate for the calculated k_c was in the range of values in the vertical bar, given $\theta = 0.015$, $f = 0.3$.

Considering the potential variations in dissociation constants (K_d values), EC_{50} values, and k_i that are factors in the calculation of k_c, it is appropriate to regard the results of the calculation of k_c as an estimate of a minimum value for k_c, and to place the order of magntidue for k_c at a round figure of $k_c = 100$/min.

Calculation of probability of activation per encounter

Knowing the encounter frequency k_c enables us to calculate the product $k\tau$, in order to examine the probability that a 100% occupied receptor will activate G during an encounter of duration τ. Since

$$k_c = k_a/\theta k\tau$$

then the product $k\tau$ can be calculated from any point on the normal concentration/response (fractional activation, f) curve, given $k_a = k_i f/(1-f)$ and given a value for k_i. Take the normal response to epinephrine at a concentration equal for the EC_{50}, 10 nM. At this concentration the activation rate k_a is equal to the inactivation rate k_i. Given the binding $K_d = 2\ \mu$M, then

$$\theta = 10\text{ nM}/(10\text{ nM} + 2\ \mu\text{M}) = 0.005$$

Rearranging from above, and using (for example) a minimum k_i and a maximum k_c: $k_i = 1.0$/min and $k_c = 90$/min:

$$k\tau = k_a/(\theta k_c) = 1.0/\text{min}/(0.005 \times 90/\text{min}) = 2.2$$

If a receptor is 100% occupied by epinephrine during an encounter, then the probability of cyclase activation during the encounter is given by

$$1 - e^{-k\tau} = 1 - e^{-2.2} = 0.89$$

Thus, there is a high probability (0.89) that an encounter between one G and one receptor which is occupied by epinephrine for the duration of the encounter will result in activation of G.

Comparison: Advantage of high agonist binding frequency ($t_m < \tau$) for an efficient agonist

Given such a model for the relationship between occupancy and activation, a notable prediction from it is that for a given efficiency (k) there will always be an "advantage" to high binding frequency (short lifetime of the receptor–agonist complex) with respect to the extent of activation per extent of agonist occupancy of receptors. Specifically, with respect to the equations derived above, at any given k the activation probability for a rapidly dissociating agonist ($1 - e^{-\theta k\tau}$) is always greater than the activation probability for a slowly dissociating agonist [$\theta(1 - e^{-k\tau})$]. The difference between the two expressions as a function of θ and for assigned values of the product $k\tau$ is shown in Fig. 5 and associated Table 1. In case A, the activation for one encounter is calculated for a "strong" (efficient) agonist (a) and for a "weak" (inefficient) agonist (b). This is compared to the net activation in a second case, B, in which the same net encounter duration is distributed among four encounters rather than one. As one would expect, for the strong agonist the number of activations is greatly increased. For the weak agonist, the number of activations is increased only slightly. For both agonists a and b, then, there will always be a greater extent of activation when occupancy is distributed among a larger group of receptors. The extent to which the two cases of occupancy distribution differ (i.e. the extent to which high binding frequency is advantageous) depends, however, entirely on k. If k is low, then there is no significant increase in activation due to high binding frequency; that is, if the probability of activation during one entire encounter is low to begin with, then there is little advantage to "switching" to another encounter, even were it ensured that loss of occupancy at one receptor within an encounter is replaced by occupancy at another receptor also involved in an encounter.

The illustration is not meant to imply that for a given binding frequency the binding frequency of an agonist is the sole determinant of the number of activations per occupied receptor per unit time. Rather, it shows that in a comparison of two agonists with the same efficiency (k), the agonist with the shorter receptor-bound half-life will be a better agonist per degree of occupancy, *if* the combination

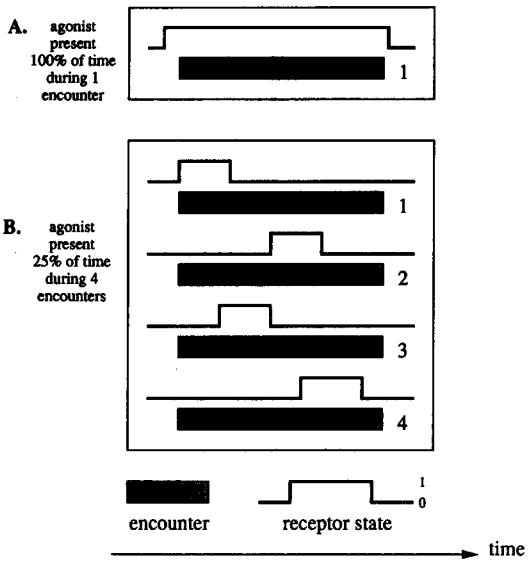

Fig. 5. Example of the theoretical activation of cyclase predicted by the encounter coupling model for two cases of the relationship of occupancy to the encounter duration: (A) agonist present during entire encounter; and (B) agonist present 25% of the time during four encounters. As in Fig. 1, the receptor state is represented as a binary variable: 0 = unbound, 1 = bound to agonist. The numbers presented in the accompanying Table 1 show the net number of activations obtained in conditions A and B for two example values for the efficiency rate constant (k) of the agonist–receptor complex: weak agonist: $k = 0.1$/unit time; and strong agonist: $k = 6$/unit time. If conditions A and B are on the same time scale, then the number of activations for the strong agonist is heavily dependent on the rate of redistribution of occupancy among a larger receptor population, whereas the number of activations for the weak agonist is not heavily dependent on the rate of redistribution of occupancy. These numbers simply illustrate for the encounter coupling scheme that (1) there will always be greater activation when agonist activity is distributed among greater numbers of encounters per unit time, which can occur by the distribution of occupancy to greater numbers of receptors per unit time, and (2) the magnitude of the difference in activation between the two cases may, however, not be significant when k is low (i.e. when the receptor–agonist complex is inefficient at activation).

Table 1. Comparison of G activations per degree of occupancy for four combinations of agonist binding frequency and agonist efficiency, as illustrated in Fig. 5

Number of encounters	Agonist	k	k/n	Number of activations
(A) $n = 1$	a	6.0	6.0	0.998
	b	0.1	0.1	0.095
(B) $n = 4$	a	6.0	1.5	3.1
	b	0.1	0.025	0.099

Case A is with agonist present 100% of the time during one encounter. Case B is with agonist present 25% of the time during four encounters. The net activations of G are calculated in each case for two agonists classified according to their efficiencies (a = "strong", b = "weak"). In this illustration, the number of activations is a function of the efficiency, k, and the number of encounters, n:

$$\text{number of activations} = n\,(1 - e^{-k/n})$$

of variables of half-lives, encounter times and encounter frequencies are such that the higher binding frequency agonist achieves a greater number of encounters between bound R and G per unit time. In the illustration we are using a 4-fold increase in the number of effective encounters per unit time matched to a correspondingly shorter (1/4) agonist–receptor lifetime as the only variable in two conditions comparing the result for a given k, but this is not accounting for the number of cycles of agonist binding and unbinding that occur with receptors that are *not* engaged in an encounter. Thus, the factor increase in activations per occupancy is a more complicated function of the binding frequency than is given in the illustration.

Relationship of the encounter model to the collision coupling model

The model by Cassel and coworkers for G protein coupling activity [7] predicts a fractional cyclase activation (fraction of maximum, f) in response to an activation rate k_a according to

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

where k_i is the cyclase inactivation rate (kinetically equivalent to the GTPase activity of the GTP-binding protein). In the collision coupling model [1, 4], the rate of activation k_a is proportional to the concentration of agonist–receptor complex, [AR], which is, in turn, proportional to receptor occupancy θ:

$$k_a \propto [\mathrm{AR}] \propto \theta$$

In the encounter model, k_a is shown to be the product of the encounter frequency (n/t), the receptor occupancy (θ), the rate constant for activation during an encounter (k) and the encounter time (τ) when the binding frequency is high:

$$k_a = (n/t)\ \theta\ k\tau$$

Thus, in both the collision coupling model and the encounter model the rate constant for cyclase activation is proportional to the receptor occupancy θ for the case in which the binding frequency is high and given a fixed total concentration of receptors. The rate of activation is not necessarily proportional to the concentration of bound receptors, however, since encounter frequency is dependent on the total number of receptors, and any given concentration of bound receptors can be theoretically achieved by an infinite combination of occupancy and receptor number.

DISCUSSION

A characteristic of β-adrenergic receptor agonism in the intact S49 cell is that the resultant activity of adenylate cyclase (the rate of cAMP generation) can be nearly maximal while receptor occupancy by agonist is well below saturation. The collision coupling model of Tolkovsky and Levitzki [4] provides a mechanistic and quantitative explanation for this relationship between receptor occupancy and cyclase response. The essential aspect of the collision coupling model was the assertion that any

G protein (kinetically coupled to cyclase) has access to numerous receptors, and that the inactivation occurred by means that are independent of the continued presence of any receptor, occupied or unoccupied. It is the relative disparity between the rates of the independent activation and inactivation processes that enables the signal of a low number of occupied receptors to be amplified to a relatively high cyclase activity. Thus, it was proposed that the mobility of receptors is apparently such that collisions between receptor and G occur with a frequency that is sufficient to maintain G in its active state, given the relative slowness of G inactivation. The model assumes that the *rate* of activation of cyclase is proportional to the number of occupied receptors, as was supported by measurement of adenylate cyclase activation in turkey erythrocytes under conditions of high occupancy of a reduced receptor number (down to *ca.* 10% of the constitutive number of receptors), where the reduction was achieved by a pretreatment with irreversible blocking agents [4].

In a previous paper [3] we used an alternative technique involving a relatively long-lived antagonist (propranolol) to reduce the effective concentration of receptors down to 1.5% of the total receptor number. Measurement of the cyclase activity in S49 cells under conditions of high occupancy by epinephrine of the diminished number of receptors showed that the rate of activation of cyclase is not strictly proportional to the concentration of agonist bound receptors under all circumstances. Specifically, high occupancy of 1.5% of the receptors resulted in a rate of cyclase activation that was substantially less than that observed for 1.5% occupancy of the entire receptor population. The simplest explanation for this result can be derived from the basic tenets of collision coupling but with a modification to allow for each "collision" to have some finite duration. If a collision has essentially zero duration, then the same number of collisions per unit time between occupied receptors and G will occur irrespective of how the concentration of occupied receptors is obtained, whether by unrestricted sharing (or turnover) of occupancy among a relatively large receptor population (as is the case in the normal concentration vs occupancy curve), or by a fixed number of completely occupied receptors. In either case a collision will occur with a receptor that is strictly in either the bound state or the unoccupied state, with the probability of being in the bound state equal to the overall receptor occupancy by agonist. Envisioning a "collision" as being rather an interaction of non-zero duration (an "encounter"), then the possibility exists that the receptor can change state with respect to agonist occupancy during that time period. In that circumstance, the rate at which occupancy turnover occurs (the agonist binding frequency) could have some impact on the rate of G protein activation per occupied receptor. This would make the relationship between the rate of cyclase activation and the concentration of occupied receptors more complex than a strict proportionality.

A potential consequence of this for G/adenylate cyclase activation by agonist-bound β-adrenergic receptors is readily seen in qualitative terms: if G were activated rapidly during such an interaction with a receptor–agonist complex, then any subsequent collisions between the same receptor and G would be non-productive, given that the G does not require the continued presence of the receptor to remain active, and given that the G activity is so relatively long-lived. Whether the rate of agonist occupancy redistribution contributes to the net rate of G activation should depend on the lifetime of an encounter relative to the lifetime of the agonist–receptor complex, and the frequency with which encounters occur. Here we have developed the concept of encounter coupling and the relationship of these variables in more formal detail. A general result is that we have shown from a theoretical standpoint how these factors can influence the activation rate within the simplest possible encounter coupling scheme. A specific result is that we have calculated the apparent encounter frequency for β-adrenergic receptor/G interactions in the S49 cell.

As described above, the encounter frequency was calculated using the results of an experiment in which the rate of adenylate cyclase activation depended mainly on the encounter frequency. The estimation of the encounter frequency from the measured activation rate required, in addition, a measurement of the inactivation rate constant for activated G/adenylate cyclase. Approaches to the determination of the rate constants for activation and inactivation of adenylate cyclase in cell-free systems have been published by Cassel and coworkers [6, 7]. The method consists essentially of determining the time delay in the change in the rate of synthesis of cAMP on an appropriate change in stimulation. Cassel *et al.* [6, 7] showed that the rate constants for adenylate cyclase activation and inactivation could be determined graphically from time course data by equating the rate constant for the process with $1/t$(delay). The effective rate constant for the measured overall activation process of adenylate cyclase is given by $(k_a + k_i)$, where k_a and k_i are the rate constants for the individual activation and inactivation processes. In the present case we determined the effective rate constant $(k_a + k_i)$ at a single concentration of agonist, and calculated k_i by relating k_a and k_i using the agonist EC_{50}.

The value $k_i = 1.4$/min was much lower than that determined for adenylate cyclase in turkey erythrocyte membranes (15/min) [7]. It corresponds well, however, with the GTPase activity measured by Brandt *et al.* [13] in their reconstituted system. Their published rate of hydrolysis of 1 mol of GTP hydrolyzed/min/(mol of G_s) corresponds to a k_i of 1/min. The range of values for k_i obtained here is relatively small, however, and for the purposes intended, the important aspect of the k_i measurement is not derived from its precision, but rather from confidence in its order of magnitude, since that enables the order of magnitude for the encounter frequency between receptors and G to be determined. Although the rate constant for cyclase inactivation derived from the cAMP accumulation experiments is comparable to measurements in other systems of the relatively slow GTPase activity of G, our measurement of the cyclase activation is only an indirect measurement of the activity of G. Although

the process of cyclase inactivation may not be solely and specifically equivalent to the process of GTP hydrolysis, we have assumed that the kinetics for the process of inactivation can be adequately modeled by a first-order rate constant, and, based on previous evidence, we have assumed in our description of the system that G activity and cyclase activity are kinetically equivalent [8].

An additional and independent perspective on the value for k_i is related to diffusion coefficients of receptors. The results shown in Fig. 3 have been analyzed previously to estimate the minimum diffusion coefficient for β-adrenergic receptors in the S49 cell [3]. In essence, the analysis simply evaluated the minimum rate that receptors would have to move around the cell surface (in terms of the diffusion coefficient, D) in order for a fixed number of fully-occupied receptors to effectively "cover" the cell surface and keep cyclase activated to the measured extent given the rate of cyclase inactivation. Using the value $k_i = 1.4$/min as a given value, a minimum value for D was estimated to be on the order of 0.01 μm^2/sec. This value for D is only slightly less than the value that has been reported for actual measurements of the mobility of tetramethylrhodamine-labeled G protein $\beta\gamma$ subunits in NG-108-15 cells (on the order of 0.02 μm^2/sec) made by Kwon *et al.* [14]. Assuming that the diffusion coefficient for β-adrenergic receptors in the S49 cell is comparable in magnitude, then the equivalence of the measured value for D in NG-108-15 cells to that predicted in S49 cells on the basis of an assumed value for k_i supports that assumption and thereby also the measurement of k_i made here.

The order of magnitude of the diffusion coefficient brings up an important point regarding the distinction between the encounter coupling model and the model developed previously by Swillens [10]. Swillens' model envisioned that interactions between a pair of molecules within a constrained environment such as the cell membrane could take place over a finite period of time and with a large number of collisions. This consideration was described in quantitative terms with defined parameters, beginning with a definition of an "encounter zone" around each adenylate cyclase. It was assumed that in this encounter zone any receptor is able to interact with and to activate the adenylate cyclase in question. Outside of the zone, it as assumed that no interaction is possible. Rates into and out of the zone are determined by the diffusion coefficients. According to such a model, the mean period of interaction is a function of the diffusion coefficients and the radius of the encounter zone. In the encounter coupling model developed here we have assumed only that a given receptor undergoes encounters that have some finite mean duration. We are not attributing the lifetime of the encounter solely to diffusion, however. If the mean lifetime of a diffusion-dependent encounter is at least as long as the mean lifetime of an epinephrine–receptor complex (which is estimated to be on the order of 0.2 sec [15]), and the diffusion coefficients are on the order of the values given above, then it can be shown that the encounter zone would need to be many times larger than the probable dimensions of a protein the size of the β-adrenergic receptor. Thus, it seems unlikely that the duration of an encounter is dependent solely on diffusion, at least not if the same diffusion rates apply as when the proteins are *not* in the vicinity of each other [14]. Although the activation of G by agonist-bound receptors must require a specific interaction, there is no independent information on the lifetime of the receptor–G complex in the presence of GTP (which is always available in the intact cell), and the lifetime of an encounter could instead be due in large part to relatively nonspecific protein–protein interactions [16]. Whatever are the forces by which the interaction between receptors and G is maintained, in the encounter coupling model presented here it is assumed simply that the duration of an encounter is finite, and that the probability of activation of adenylate cyclase is related to the duration of an encounter and to an intrinsic agonist-dependent efficiency (here labeled k) of the receptor when bound. As in Swillens' model, then, the overall rate of adenylate cyclase activation is here a function of both the probability of activation during an encounter of finite duration and also the number of encounters per unit time.

It is important to discuss in some detail some of the other assumptions that are intermediate between what the previous data show and the calculation of the encounter frequency from them. The epinephrine plus propranolol experiment (Fig. 3) compared cAMP accumulation under two conditions which contain an equal number of receptor–agonist complexes, but in one case the agonist was effectively restricted to a smaller number of receptors. In spite of the equality of the numbers of receptor–agonist complexes, the cAMP accumulation, and therefore the extent of adenylate cyclase activation, was less in the case where the number of receptors involved was reduced. Some of the specific assumptions associated with this intermediate conclusion are that propranolol acts to block receptor activity as a prototypical agonist, and that the equations describing single-affinity ligand binding apply to both agonist and antagonist on the time scale of the experiment, such that reduction in the involvement of receptors can be forced to a calculable extent. At this level of interpretation we have made no assumptions that involve any of the complexities of the coupling of receptor to G, the G cycle, any form of desensitization or effects on cAMP hydrolysis rates; we have simply demonstrated that cAMP accumulation is affected by the time dependence of the distribution of receptor occupancy by agonist. This experimental finding was then interpreted in terms of the collision coupling model with one changed assumption. In the collision coupling model as originally formulated it was supposed that G protein/catalytic unit complexes interacted with a large number of receptors per short unit time. This was demonstrated experimentally [4], as was the important assumption that the G protein and the catalytic unit were precoupled at least in the kinetic sense [8]. What had not been demonstrated independently (but was assumed implicitly) was that collisions were of short duration. Were this assumption correct for our experimental system, then there should have been no difference in cyclase

activation under the experimental conditions we used in the presence and absence of propranolol. The one assumption we have made in this explanation that is different from the classic collision coupling model is that there is a finite duration for encounters between receptor and G, and that the time scale for the mean duration of encounters is comparable to the dissociation time for epinephrine. The detailed exposition of the evidence for this has been published previously [3].

The encounter coupling model developed here shows how agonist binding frequency can be a factor in the relationship of cyclase activation to receptor occupancy, given a finite duration (viz. a duration on the order of the receptor–agonist complex lifetime) for the interaction between receptor and G. In the illustration in Fig. 5 it is shown, for instance, that given a high agonist efficiency, two binding cycles involving two receptors can potentially activate a greater number of G proteins than can one binding cycle of twice the duration. This interpretation of the interrelatedness of intrinsic efficiency and the binding frequency as factors that influence the net extent of G activation per degree of receptor occupancy has been supported by other experiments, using a number of different agonists, in which the potential contributions of agonist binding frequency to the cyclase activation rate were examined using the technique of blocking receptors with propranolol [9].

An additional perspective on this interpretation is the question of what constitutes "high" binding frequency in the context of this model, and whether such binding frequencies are realistic ones. This is essentially a question of determining the rate constants for agonist association and dissociation, and the receptor–agonist complex lifetime. The required association rate constant for epinephrine in the intact S49 cell has been calculated to be on the order of 10^8/M/min, with a corresponding epinephrine–receptor complex lifetime on the order of 0.2 sec [15]. This value for the association rate constant is essentially the same as that for propranolol binding to intact S49 cells (unpublished data) and that for isoproterenol binding to polymorphonuclear leukocytes [17]. Thus, the constants associated with the binding frequency that are necessary for an effect of binding frequency on G activation rates according to this model do not appear to be of an unusual magnitude.

It is useful to consider these results in a broad context of the concept of "spare" receptors in general receptor-mediated signal transduction systems. Prior to the establishment of techniques whereby receptor occupancy by agonist could be measured reliably, it was supposed that the fractional cellular response to a given concentration of an agonist was proportional to the fractional receptor occupancy [18]. A landmark paper by Stephenson [19] provided evidence that this was not the case, and that the fractional cellular response could be obtained with a lesser fractional receptor occupancy depending on the agonist efficacy. This has come to be known as the "spare" receptor theory, and numerous receptor-mediated response systems are said to exhibit "spare receptors" on the basis of the fact that the concentration of agonist which elicits a 50% response is less than the concentration of agonist which results in 50% receptor occupancy (i.e. $EC_{50} < K_d$) [20]. That description can be somewhat misleading, however, with respect to making the essential distinction between receptor occupancy and the absolute numbers of receptors involved in signal transduction [20]. For example, whereas 30% receptor occupancy means precisely that 100% of the receptors are occupied 30% of the time although only 30% of the receptors are occupied at any one time, the term "spare" receptors, when used to convey the meaning that a greater fractional level of response can be obtained at this level of occupancy, would imply (as the terminology of Stephenson did imply) that 70% of the receptors are not involved in, and are not necessary for, the activation of the response. Whether the turnover of occupancy is potentially relevant in any system depends in part on the time scale over which the turnover is significant compared to the time scale over which the response is measured. In the S49 cell, the binding frequency of epinephrine appears to be rapid enough such that even at relatively low overall receptor occupancy, most of the receptors are involved with epinephrine within a matter of a few seconds [15], whereas the cyclase response is typically measured over a time frame of many seconds. It should be noted that the collision coupling model does not ignore the concept of occupancy turnover, but rather asserts that it should not be a factor in the relationship between receptor occupancy and response. In fact, the contribution of agonist binding frequency to the concentration versus response curves for β-adrenergic agonists becomes apparent only under a limited range of conditions that have been demonstrated only recently [3, 9].

The use of the term "binding frequency" in relation to signal transduction can bring to mind Paton's "rate theory" for receptor-mediated agonist action [21]. It is important therefore to make explicit the distinction between rate theory and encounter coupling, and to emphasize that the encounter coupling concept is not an assertion of the rate theory. The rate theory postulated that an agonist transmitted its action in proportion to its rate of binding to its receptor. In the encounter coupling model, an agonist is capable of transmitting its action anytime while it is bound to the receptor, but is able to do so only during periods in which the receptor is in a properly evocative position relative to G. In preceding papers [3, 15] we have shown that not only is such a position achieved only episodically (as was asserted by the collision coupling model), but that in addition each episode has some mean duration that is significant relative to the mean lifetime of the agonist–receptor complexes for some agonists. For that reason, according to the encounter coupling model, the agonist binding frequency can influence the quantitative relationship between receptor occupancy and the rate of activation of the next process in the signal transduction scheme. This is not at all the same as saying that the rate of activation is proportional to binding frequency.

It is important also to emphasize that the calculation of encounter frequency from these data

is not a form of additional evidence in support of the encounter coupling model. Rather, the analysis provides an estimation for the value of a parameter (the encounter frequency) that is essential to further quantitative assessment of the model. In addition to the estimation of the encounter frequency, further tests of the model in this system require an estimate for the encounter duration, such that the numerical predictions of the model for cyclase activation rates can be compared to experiments using other agonists with different agonist–receptor complex lifetimes. A model that is both more general and more complete than the one presented here will also include the rate constants for agonist association and dissociation as explicit variables. Whether the relationship of agonist binding frequency to G activation as explained by encounter coupling is observable in systems other than the S49 cell remains to be determined.

According to this analysis, receptor/G interactions occur with a frequency in the S49 cell that is on the order of 100/min. Prior to this analysis (i.e. without any interpretations of the data in terms of encounters and the extended collision/encounter coupling model), estimates of a value for the rate of receptor/G interactions that are an order of magnitude greater may not have seemed unrealistic, and such estimates would have required assumptions about the ratio of the two species and their diffusion coefficients. Here the interaction rate has been derived from experiments that have used only easily measurable ligand dissociation constants, the agonist EC_{50} and the cyclase inactivation rate constant. As shown above, however, even only 100 encounters per minute (i.e. the access of any one G to this many receptors per minute) can easily account for the relationship between agonist concentration, binding and response wherein near maximal cyclase activation can be obtained at low receptor occupancy, given the relative slowness of G inactivation.

In summary, the curve for the fractional activity of adenylate cyclase in intact cells as a function of agonist concentration is invariably left-shifted from the receptor occupancy curve ($EC_{50} < K_d$). This relationship between activity and occupancy can be explained by the assumption that the rate of cyclase activation is proportional to the concentration of agonist-bound receptors (the collision coupling model). Experiments in which the number of receptors was reduced drastically have shown that this assumption is not correct under all circumstances, and the experiments revealed an otherwise-hidden component of the relationship between receptor occupancy and cyclase activity that can be attributed to the agonist binding frequency. The influence of agonist binding frequency can be explained in principle with the assumption that each interaction between receptors and G has some finite duration (the encounter coupling model). Using the encounter coupling model, the apparent frequency of such interactions (encounters) has been estimated to be on the order of 100/min in the intact S49 cell.

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