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Comparisons of the Combined Contributions of Agonist Binding Frequency and Intrinsic Efficiency to Receptor-Mediated Activation of Adenylate Cyclase

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SUMMARY

A given overall level of β-adrenergic receptor occupancy by agonist can involve either high or low turnover of occupancy with respect to individual receptors, depending on the binding properties of the particular agonist. It was recently demonstrated that, for epinephrine-stimulated adenylate cyclase activation in the S49 cell, a portion of the separation between the β-adrenergic receptor binding curve and the cyclase response curves is dependent on high occupancy turnover (high binding frequency). By involving a larger number of receptors within a short period of time than are bound at any one instant, the effect of high binding frequency is to increase the rate of GTP-binding protein/adenylate cyclase activation over the rate that is observed when the mobility of the number of receptors occupied at any given instant is the rate-limiting factor. This phenomenon contributes to the normal dose-response curve for epinephrine, according to our analysis, but only in combination with the apparent high efficiency of the receptor in the epinephrine-bound state at cyclase activation. Here we examined the potential combination of the contributions of agonist binding frequency and intrinsic efficiency to the adenylate cyclase activation rate for four other β-adrenergic agonists (isoproterenol, zinterol, metaproterenol, and dobutamine). This was done by a comparison of the response (1-min cAMP accumulation) between a point on the normal dose versus response curve (control) with the response under conditions in which the concentration of agonist-bound receptors was identical to control but the absolute number of receptors involved in maintaining that concentration was significantly reduced. In the experiments, the majority of the receptors were blocked by the β-adrenergic antagonist propranolol, which has a relatively long occupancy half-life. The remaining receptors were occupied by agonist such that the concentration of bound receptors was identical to the control condition of low occupancy of the full complement of receptors in the absence of antagonist. Compared with control, the experimental condition was one in which agonist occupancy turnover was inhibited and the potential contribution of agonist binding frequency as a factor contributing to the cyclase activation rate was greatly reduced (producing a point on the receptor mobility-limited dose versus response curve). Isoproterenol and metaproterenol show evidence that their binding frequencies and the efficiency of the receptor when bound to them are of such a combination that the normal dose-response curves for these agonists contain a component that is dependent on the binding frequency. No effect of binding frequency on activation is observed for dobutamine and zinterol, attributable to either low binding frequency, low efficiency, or both. These results are consistent with predictions based on previous experiments using epinephrine; by this analysis, the results reflect properties not only of specific agonists but also of the interaction between receptor and GTP-binding protein that are important in understanding and quantitating the relationship between β-adrenergic receptor binding and adenylate cyclase response in the S49 cell.

β-Adrenergic receptor-mediated activation of adenylate cyclase exhibits an agonist-specific separation between the dose-response curve (characterized by the EC_{50}, the concentration required for 50% activation) and the dose-binding curve (characterized by the K_d, the concentration required for 50% receptor occupancy), such that cyclase activity can be near-maximal when receptor occupancy is still quite low ($EC_{50} \ll K_d$). This separation is in large part due to the mobility of receptors in the membrane and the ability of one receptor to activate more than one cyclase (1). This separation (the PSR, K_d/EC_{50}) (2) is predicted by the collision coupling model of Tolkovsky and Levitzki (1), which assumes that the rate of activation is proportional to the concentration of bound receptors that are mobile catalysts for adenylate cyclase activation. There is much evidence supporting the basic truthfulness of this model for the coupling of receptors to cyclase, e.g., nonlinearity between response and receptor number (1) and the proportionality (at high receptor occupancy) of activation rate and membrane fluidity (3).

We recently demonstrated circumstances in epinephrine-

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ABBREVIATIONS: PSR, pharmacological shift ratio; G protein, GTP-binding protein; PDE, phosphodiesterase.

stimulated S49 cells in which the rate of cyclase activation is not proportional to the receptor occupancy by agonist or the concentration of agonist-bound receptors (4). We used, as a starting point, the prediction of the collision coupling model for the response to agonist (A) in the presence of a competitive inhibitor (I), equivalent to the Cheng-Prusoff relation (5):

$$\text{predicted fractional response } f = \frac{[A]}{[A] + \mathrm{EC}_{50}\left(1 + \frac{[I]}{K_{d_I}}\right)}$$

where K_{d_I} is the dissociation constant for I. In a series of experiments with epinephrine (A) and propranolol (I), both $[A]$ and $[I]$ were increased in such a way as to keep the right side of the equation constant and such that a single fixed concentration of A-bound receptors was maintained. This resulted in a decreased response in adenylate cyclase activation [up to 50%, compared with 30 nM epinephrine control (EC_{50} = 10 nM)] as $[A]$ and $[I]$ were increased. The reason for the disparity between the observed response and the predictions of collision coupling and the Cheng-Prusoff relation is that these relations do not take into account any potential role of ligand binding frequency or of the number of receptors involved with agonist per unit time; when A and I are epinephrine and propranolol, the large disparity in the half-lives of the ligand-receptor complexes bound to epinephrine (short $t_{1/2}$) versus those bound to propranolol (long $t_{1/2}$) is such that, under this protocol, a fixed concentration of bound receptors can be isolated to a small number of receptors (i.e, the absolute number of receptors participating, per unit time, in maintaining occupancy by A becomes smaller), rather than being shared among the population of receptors. As $[A]$ and $[I]$ were increased, conditions under this protocol approached one in which receptor mobility alone was responsible for the rate of encounters between receptors and adenylate cyclase and, hence, the adenylate cyclase activation rate. Diminishing the contribution of epinephrine binding frequency led to a decrease in response.

This result demonstrated that, for epinephrine, part of the PSR is attributable to its binding frequency (the rate at which individual receptors bind and unbind), which is apparently high enough to ensure that separated regions of the cell surface experience some period of agonist-bound receptor activity per small unit of time. This means, for instance, that a greater rate of G protein/cyclase activation is observed for 1% agonist occupancy of 2000 receptors, compared with that for 20 receptors occupied by agonist 100% of the time, even though in both cases 20 receptors are occupied by agonist at any given instant.

The simplest interpretation of the contribution of agonist binding frequency to the rate of G activation is that it is due to a combination of two factors; (a) the interaction between individual receptors and G protein has a mean lifetime that is greater than the mean lifetime of the epinephrine-receptor complex and (b) epinephrine-bound receptors are very efficient at activating G protein. Because of factor a, a receptor can change occupancy state (from bound to unbound, or vice versa) during an encounter with G. Because of factor b, the statistical replacement of agonist unbinding at one site by agonist binding at another site increases the overall probability of G activation per unit time. There would be no observable effect of the lack of "switching" of occupancy among receptors were both conditions not met.

According to this interpretation, the interaction between receptors and G probably entails multiple collisions in short-order sequence (which we have called an encounter). When the rate of redistribution of occupancy is restricted, as in the mobility-limited case, it is possible that a high efficiency agonist such as epinephrine will "waste" some collisions within an encounter with G molecules that have already become activated within that encounter. Conversely, when occupancy is shared and rapidly distributed among a large population of receptors, it is less probable that any one agonist-bound receptor will waste collisions within an encounter with adenylate cyclase molecules that it has already activated, because the receptor is bound for a very short time. The extent of G/cyclase activation is thus greater for the case of high turnover of agonist occupancy.

The results in experiments with epinephrine led to the conclusion that binding frequency of epinephrine could contribute a factor of 6 to 7 to the PSR observed for epinephrine in the intact S49 cell (PSR = 200). Thus, the majority of the PSR (a factor of approximately 30) is contributed by receptor mobility. In principle, the magnitude of the separation between the K_d and the EC_{50} for the mobility-limited case would be independent of the agonist when the agonist is highly efficient. Specifically, because the rate of collisions between receptors and cyclase is probably largely independent of receptor occupancy or the agonist with which a receptor is occupied, we would predict that the mobility-limited dose-response curve for an agonist of high efficiency will be shifted to the left of the binding curve by a factor of (at most) approximately 30, because this factor is dependent only on the diffusion rates of receptors and G protein in the membrane (dictating maximum rates of interaction between the two species).

This prediction and the general applicability of this scheme for receptor-G protein interaction based on the results of experiments using epinephrine are tested here using four other β-adrenergic agonists (isoproterenol, metaproterenol, zinterol, and dobutamine), which cover the available range (excluding epinephrine) of K_d/EC_{50} ratios ($4 < PSR < 150$). We examined the extent to which the mobility-limited dose-response curve differs from the usual dose-response curve, using 1-min cAMP accumulation assays in the presence of varying amounts of agonist and the antagonist propranolol calculated to give a constant concentration of agonist-bound receptors. For agonists with a PSR of >30 (isoproterenol and metaproterenol), a contribution of binding frequency to cyclase activation was observed; moreover, the extent of the contribution correlated, as predicted, with the extent to which the PSR was greater than 30. A contribution of binding frequency was not observed for zinterol and dobutamine, consistent with the expectation that no such contribution is possible when binding frequency is low (as is probably the case for zinterol) or when the intrinsic efficiency is low (as is probably the case for dobutamine). Thus, the results obtained earlier using epinephrine are not a reflection of some peculiar property of epinephrine or its interaction with receptors but reflect a general phenomenon of receptor-G protein interaction in this cell. The results can be interpreted in terms of a collision coupling model modified simply to include collision sequences (encounters) between individual receptors and G proteins that have some nonzero duration.

Experimental Procedures

Materials. (−)-Epinephrine bitartrate, metaproterenol, zinterol, dobutamine, and (±)-propranolol were purchased from Sigma; metoprolol

(5 mg/5 ml of H_2O) was purchased from Geigy; and [8-^{3}H]adenine (17 Ci/mmol), [8-^{14}C]ATP (500 mCi/mmol), and [8-^{14}C]AMP were purchased from ICN.

Cell culture. S49 murine lymphoma cells were kindly provided by Dr. Henry Bourne (University of California, San Francisco). Cells were grown at 37° in 1-liter roller bottles with Dulbecco's modified Eagle's medium (GIBCO) plus antibiotics and 5% horse serum. Cell density was kept between 1.5 and 2.0 × 10^6 cells/ml by daily addition of fresh medium.

cAMP accumulation experiments. Experiments measuring cAMP accumulation were conducted as previously described (6). Cells were washed in serum-free Dulbecco's modified Eagle's medium, resuspended at a density of 20 × 10^6 cells/ml, and incubated for 60 min at 37° in medium containing 10 mCi of [^{3}H]adenine/ml ("prelabeling" of ATP pool). Adenine remaining in the medium was removed by washing, and the cells were incubated in fresh medium for 30 min at 37°. After agonist addition, aliquots were removed and centrifuged; medium was discarded by aspiration. Cell response was stopped after 1 min by addition of 5% trichloroacetic acid. Precipitated protein was removed by centrifugation before the liquid extracts were fractionated on Dowex 50 and alumina columns. [^{14}C]ATP and [^{14}C]cAMP were used to monitor recovery. Accumulation of cAMP during 1 min was measured as percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP:

$$\%\ \text{of conversion} = \frac{[^3\text{H}]\text{cAMP}}{[^3\text{H}]\text{cAMP} + [^3\text{H}]\text{ATP}}$$

Experiments limiting receptor participation by addition of antagonist. The control experiment was a 1-min assay of cAMP accumulation during stimulation by a concentration of agonist (A) that was chosen to give >50% activation (f), given its approximate EC_{50} using

$$f = \frac{[A]}{[A] + EC_{50}}$$

Occupancy (θ) of β-adrenergic receptors for control was calculated, given K_{d_A}, by

$$\theta_A = \frac{[A]}{[A] + K_{d_A}}$$

In subsequent experiments, cAMP accumulation was measured for the same agonist-receptor occupancy but in the presence of increasing concentrations of antagonist (where agonist occupancy was maintained by increasing agonist concentrations as well). The approach can be viewed from the standpoint of the Cheng-Prusoff relation (5):

$$\text{predicted fractional response} = \frac{[A]}{[A] + EC_{50}\left(1 + \frac{[I]}{K_{d_I}}\right)}$$

from which, for a given $[A]$, $[I]$ was calculated in order to maintain the right side of the equation constant and equal to the control (where $[I] = 0$). Letting $[A]$ for control = $[A]_0$, the relationship between $[I]$ and $[A]$ is given by

$$[I] = K_{d_I}\left(\frac{[A]}{[A]_0} - 1\right)$$

This calculation gives $[I]$ that keeps θ_A constant, and equal to θ_A for control, for any $[A] > [A]_0$.

Split addition versus preaddition of antagonist. The problem of achieving and/or maintaining constant agonist and antagonist occupancy during the course of the experiment, which arises from the disparity between the agonist and antagonist kinetic constants for dissociation, has been discussed previously (4). Our solution to this problem has been to use "split" additions of antagonist as follows:

Given the desired final concentration of antagonist I in the presence of agonist A, the steady state occupancy for I is given by:

$$\theta_I = \frac{\frac{[I]}{K_{d_I}}}{1 + \frac{[A]}{K_{d_A}} + \frac{[I]}{K_{d_I}}}$$

Before the addition of agonist as a competing ligand, this occupancy for antagonist can be achieved by a concentration of I (= $[I]_0$) satisfying the relation

$$\theta_I = \frac{[I]_0}{[I]_0 + K_{d_I}}$$

i.e.,

$$[I]_0 = \frac{K_{d_I}\theta_I}{1 - \theta_I}$$

This amount of antagonist, sufficient to occupy the same fraction of receptors in the absence of agonist as is desired when agonist is added, was added 6 min before the beginning of the assay time. At the concentrations of antagonist used in these experiments, 6 min was sufficient for equilibrium to be obtained. To begin the assay, the required amount of agonist was added together with the calculated additional amount of antagonist ($[I] - [I]_0$) necessary to maintain the receptor occupancy by antagonist in the presence of the competing agonist. Using this protocol of split additions of antagonist, a steady state in receptor occupancy for both antagonist and agonist was reached within 90% within 5 sec after the start of the assay for all the combinations of agonists and antagonists and the concentrations used in these experiments.

Calculation of activation rate from cAMP accumulation. Maximum activity V_{max} for cyclase was calculated from the control experiment activity v

$$V_{max} = v\left(1 + \frac{EC_{50}}{[A]}\right)$$

or measured directly in an experiment using a saturating concentration of agonist. For subsequent points, the fractional activity f was calculated by

$$f = \frac{v}{V_{max}}$$

Because activity represents a steady state between processes of activation and inactivation (7, 8), then

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

where k_a is the activation rate constant and k_i is the inactivation rate constant.

These experiments (measurement of f after a 1-min exposure to agonist) were conducted for four β-adrenergic agonists, using the following values for K_d and EC_{50}: isoproterenol, K_d = 300 nM, EC_{50} = 2 nM, PSR = 150; metaproterenol, K_d = 8 μM, EC_{50} = 200 nM, PSR = 40; zinterol, K_d = 20 nM, EC_{50} = 2 nM, PSR = 10; and dobutamine, K_d = 3 μM, EC_{50} = 700 nM, PSR = 4.

Results

PSRs in the intact S49 cell. For the purpose of making clear the rationale and principles involved in this investigation, it is useful first to present data for normal dose versus response curves of β-adrenergic agonists and to examine in detail the relationship between receptor binding and response and its explanation in terms of collision coupling and the G protein activation/inactivation cycle. Examples of dose versus response curves in the intact S49 cell are shown in Fig. 1 for seven

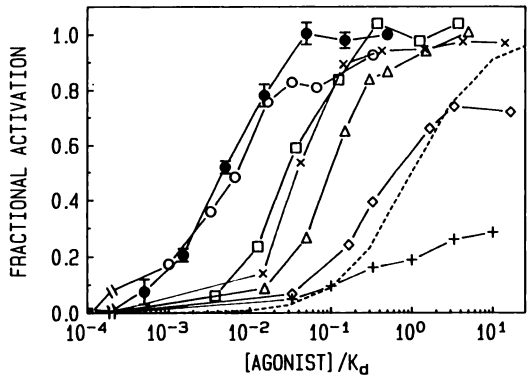


Fig. 1. Examples of dose versus response curves for epinephrine (●), isoproterenol (○), salbutamol (□), metaproterenol (×), zinterol (△), dobutamine (◇), and ephedrine (+). Response is 1-min cAMP accumulation (percentage of conversion), which is proportional to the maximum adenylate cyclase activity (rate of cAMP generation) at each dose (6). The response curve for each agonist has been normalized to the maximum response obtained using epinephrine. In order to show the relative PSR values for these agonists, the doses for each agonist have been scaled to the dissociation constant for that agonist, K_d. These agonists cover the range of PSRs (PSR = K_d/EC_{50}) for this cell, where PSR characterizes the separation between the response curve and the binding curve (- - -). The K_d values and PSRs are given in Table 1. K_d was measured for intact S49 cells using the 1-min nonequilibrium technique of Toews *et al.* (9) The *error bars*, shown only for epinephrine, are representative of the scale of the standard errors of the mean cAMP accumulation calculated from triplicate determinations under each condition. The binding curve is the theoretical curve for the fraction of receptors bound to agonist (θ) as a function of agonist concentration [θ = [agonist]/([agonist] + K_d)], ranging from 0 to 1.

agonists (epinephrine, isoproterenol, metaproterenol, dobutamine, zinterol, ephedrine, and salbutamol), where the response is cAMP accumulation in 1 min, which is proportional to the maximum rate of cAMP generation (the cyclase activity) at that dose (6). The dose for each agonist [A] has been scaled relative to its measured K_d (the dissociation constant for agonist binding to receptors), in order to normalize the response curves with respect to receptor occupancy. The theoretical agonist binding curve for fractional receptor occupancy θ [θ = [A]/([A] + K_d)] is also shown in Fig. 1 as a function of the scaled agonist concentration. Emperically, the relationship in such experiments between the dose and the cyclase activation for any agonist is given by:

$$f = \frac{v}{V_{max}} = \frac{[A]}{[A] + EC_{50}}$$

where EC_{50} is the concentration of agonist required for half-maximal response and V_{max} is the maximum rate of cAMP generation that can be obtained with that agonist. The PSR, K_d/EC_{50} quantifies the separation between the binding curve (characterized by K_d) and the response curve (characterized by EC_{50}) (2). Comparing two agonists, then, the agonist with higher PSR requires a lesser extent of receptor occupancy than is required to elicit the same degree of response by the agonist with the lower PSR. For an agonist with a high PSR, such as epinephrine, only a very low receptor occupancy is required for near-maximal activation of adenylate cyclase.

The fundamental explanation for the relationship between binding and response begins with the activation cycle for adenylate cyclase demonstrated by Cassel and Selinger (7), which is summarized by the following simplest scheme for independent processes for activation and inactivation:

$$\text{inactive } G_s/\text{cyclase} \underset{k_i}{\overset{k_a}{\rightleftarrows}} \text{active } G_s/\text{cyclase}$$

where k_a is the rate constant for G/cyclase activation due to receptor activity and k_i is the rate constant for G/cyclase inactivation (the GTPase activity rate constant), which is independent of receptor activity (and is relatively slow). Both the collision coupling model of Tolkovsky and Levitzki (1) and the more complex models of Swillens (10) use this scheme to derive a quantitative relationship between K_d, EC_{50}, and the rate constants for activation and inactivation of the cyclase (k_a and k_i, respectively, in the diagram above), by assuming that k_a is proportional to the concentration of agonist-bound receptors ($k_a = k[AR]$). The relationship is derived by equating k_a to $k[AR]$ in the expression for the fractional activation of cyclase at steady state derived from the Cassel and Selinger scheme.

In the steady state of cyclase activation, the rate of activation $(1 - f)k_a$ is equal to the rate of inactivation ($k_i f$), where f is the fraction of cyclase activated (and is equivalent to v/V, where v is the actual measured activity and V is the potential activity with 100% of the cyclase active). Equating these and rearranging:

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

The agonist-receptor concentration [AR] is given by the fractional receptor occupancy θ multiplied by the total receptor concentration R_T; [AR] = R_T[A]/(K_d + [A]), where [A] is the concentration of agonist. Substitution of k_a by k[AR] gives, after rearrangement:

$$f = \frac{v}{V} = \frac{kR_T}{kR_T + k_i}\left(\frac{[A]}{[A] + \left(\frac{k_i}{kR_T + k_i}\right)K_d}\right)$$

A comparison with the previous equations shows that V_{max} is given by

$$V_{max} = \frac{kR_T}{kR_T + k_i} V$$

and that EC_{50} is given by

$$EC_{50} = \frac{k_i}{kR_T + k_i} K_d$$

These equations can be combined with elimination of kR_T and k_i, to give:

$$\frac{K_d}{EC_{50}} = \frac{V}{V - V_{max}}$$

The rate constant k_i depends on the GTPase activity of the G protein and is independent of the agonist. The rate constant k, on the other hand, is related to the rate at which the agonist-receptor complexes can catalyze the cyclase activation. In terms of collision coupling, k may be viewed as the product of two rate constants, one for the collision frequency (the number of collisions between receptor and G per unit time) and the second for agonist-receptor efficiency (the probability that a collision with a bound receptor results in G activation). Therefore, for more efficient agonists, k will be larger, the ratio $(kR_T + k_i)/k_i$, which is equal to the ratio K_d/EC_{50}, will be larger, and V_{max} will approach V. Tests with a number of agonists using the S49

system, as in Fig. 1, confirm the usefulness of these equations. Table 1 lists the K_d/EC_{50} ratios and the maximum rates of cAMP accumulation for the agonists shown in Fig. 1. The maximal rates of cAMP accumulation calculated from the K_d/EC_{50} ratios are shown for comparison. The predicted V_{max} and the V_{max} calculated from the model are basically identical.

The collision coupling model can thus accurately characterize the relationship between the binding and response curves; moreover, the collision coupling model also accurately predicts the relationship between the PSR and V_{max} and shows that PSR is necessarily large for full agonists and is decreased for partial agonists. These results follow from the assumption that the rate of activation of G protein/cyclase is proportional to the concentration of agonist-bound receptors, and according to collision coupling the differences in PSRs among various agonists are completely attributable to differences in the agonist efficiency k.

As described above, it has been shown, however, that for epinephrine the time dependence of the distribution of agonist occupancy also influences the PSR. This binding frequency component of the PSR is essentially "hidden" in the normal dose-response curve. The time dependence of agonist occupancy can be altered using the antagonist propranolol. In the experiments reported below, that approach was used to examine potential contributions of the turnover of agonist occupancy to cyclase activation for other β-adrenergic agonists. The agonists used in this study cover the available range of PSRs in the S49 cell, as shown in Fig. 1, excluding epinephrine, which has the highest known PSR (PSR = 200).

Receptor participation limited by a slowly dissociating antagonist, propranolol. The experiments using propranolol were designed to limit the number of receptors accessible to agonist over the time course (1 min) of the experiment, while maintaining in each case a fixed concentration of agonist-bound receptors. Because of the long half-life of the propranolol-β-adrenergic receptor complex, on the order of 150 sec, a receptor bound to propranolol at the time of addition of agonist is likely to remain bound to propranolol during the entire 1-min assay. Using propranolol under the protocol described created a condition in which receptor mobility becomes the limiting factor in adenylate cyclase activation rate.

For isoproterenol (K_d = 200–300 nM, EC_{50} = 2–3 nM, PSR = 100–150) (Table 2), the restriction of agonist occupancy by this means to a smaller number of receptors reduced the activity of adenylate cyclase, compared with control of 10 or 5 nM isoproterenol (Fig. 2A). In the presence of the highest propranolol concentration used, the 1-min cAMP accumulation was reduced to 67% (average of five experiments; range, 60–80%) of control. The same concentration of propranolol had no effect on 5 μM prostaglandin E_1-stimulated cAMP accumulation (data not shown).

There was an only slight but reproducible effect of propranolol, under this protocol, on stimulation by metaproterenol (K_d = 8 μM, EC_{50} = 200 nM, PSR = 40) (Fig. 2B). For reasons discussed in detail below, the slight magnitude of this effect was the expected result. In contrast, the presence of propranolol had no effect on 1-min cAMP accumulation stimulated by zinterol (K_d = 20 nM, EC_{50} = 2 nM, PSR = 10) or dobutamine (K_d = 3 μM, EC_{50} = 700 nM, PSR = 4) (Fig. 2, C and D).

Receptor participation in the presence of a rapidly dissociating antagonist, metoprolol. A second series of experiments was conducted with the β-adrenergic antagonist metoprolol, which can be viewed as a control for the propranolol experiments. Metoprolol is a low affinity antagonist (K_d = 240 nM), compared with propranolol, and it is, therefore, expected to have a much shorter bound-complex $t_{1/2}$ than propranolol (9, 11). Given $K_{d_{metoprolol}}$ = 240 nM and $K_{d_{propranolol}}$ = 650 pM, and assuming that $k_{association} = 4 \times 10^8$/M/min for each (the value for propranolol),[1] then the half-life for the bound state ($t_{1/2} = \ln 2/k_{association}$ (K_d) for metoprolol is $t_{1/2} < 0.5$ sec, whereas for propranolol $t_{1/2}$ = 160 sec. Although high occupancy by metoprolol can be achieved in the manner used in the propranolol experiment, metoprolol probably cannot limit the access of agonist to the full number of receptors during the 1-min assay in the same way as propranolol, because of its shorter bound half-life. As predicted on this basis, metoprolol had no effect on the ability of the fixed concentration of isoproterenol-bound receptors to activate adenylate cyclase (Fig. 3A) or on activity promoted by zinterol (Fig. 3B).

Simulations of receptor occupancy. The experiments using propranolol were designed to reduce the number of agonist-bound receptors participating per unit time in the coupling to G protein/adenylate cyclase. This effect of propranolol can be visualized by a simulation of individual receptor occupancy for the various experimental conditions, as shown in Figs. 4–7. Simulations of the bound/unbound status of individual receptors over time is shown in these figures for conditions corresponding to points shown in Fig. 2 for isoproterenol, metaproterenol, dobutamine, and zinterol. The simulation is shown for two conditions for each agonist used, first, agonist alone at the control concentration and, second, agonist plus antagonist at the highest concentrations of agonist and antagonist used. The simulations demonstrate that the addition of propranolol under this protocol can alter the distribution of receptors occupied by an agonist whose occupancy entails rapid association and dissociation. When the agonist binding frequency is relatively high, as in the case of isoproterenol, propranolol reduces the number of receptors interacting with agonist per unit time, although the concentration of bound receptors cell-wide remains identical to control conditions. Over the range of propranolol concentrations used in the isoproterenol experiment,

TABLE 1

PSR (K_d/EC_{50}) and V_{max}/V for seven β-adrenergic agonists

EC_{50} values were obtained from measurements of dose versus response curves, as in Fig. 1, where response was the cAMP accumulation during a 1-min incubation of the cells in the presence of a given concentration of agonist, and where 1-min accumulation is proportional to the maximum cyclase activity V_{max} for that concentration of agonist (6). Values for the dissociation constants K_d were obtained using the nonequilibrium method of Toews *et al.* (9) for the displacement during 1 min of ^{125}I-labeled cyanopindolol. V_{max} for each agonist is taken from the examples shown in Fig. 1, using 1-min cAMP accumulation (percentage of conversion), and each has been normalized with respect to the epinephrine-stimulated V_{max}/V. Calculated V_{max} is V_{max} predicted by collision coupling and calculated from the PSR ($R = K_d/EC_{50}$) according to $V_{max}/V = (R - 1)/R$.

Agonist	EC_{50}	K_d	K_d/EC_{50}	V_{max}/V	Calculated V_{max}/V
Ephedrine	20 μM	30 μM	1.5	0.3	0.33
Dobutamine	0.7 μM	2.8 μM	4	0.7	0.75
Zinterol	2.0 nM	20 nM	10	1	0.9
Metaproterenol	0.2 μM	8 μM	40	1	0.975
Salbutamol	8.0 nM	700 nM	90	1	0.98
Isoproterenol	2.0 nM	300 nM	150	1	0.99
Epinephrine	10 nM	2 μM	200	1	0.99

[1] R. Barber, unpublished observations.

TABLE 2
Concentrations of agonists and antagonists in Figs. 2 and 3.

Figure	K_d (M)	Concentrations (M)							$\theta_{agonist}$
2A isoproterenol	3.0×10^{-7}	1.0×10^{-8}	3.3×10^{-8}	1.0×10^{-7}	3.3×10^{-7}	1.0×10^{-6}	3.3×10^{-6}		0.032
propranolol	6.5×10^{-10}	0	1.5×10^{-9}	5.8×10^{-9}	2.1×10^{-8}	6.4×10^{-8}	2.2×10^{-7}		
2B metaproterenol	8.0×10^{-6}	6.0×10^{-7}	2.0×10^{-6}	6.0×10^{-6}	2.0×10^{-5}	6.0×10^{-5}	1.0×10^{-4}		0.068
propranolol	6.5×10^{-10}	0	1.5×10^{-9}	5.9×10^{-9}	2.1×10^{-8}	6.4×10^{-8}	1.1×10^{-7}		
2C dobutamine	3.0×10^{-6}	3.0×10^{-6}	1.0×10^{-5}	3.0×10^{-5}	1.0×10^{-4}	3.0×10^{-4}			0.51
propranolol	6.5×10^{-10}	0	1.5×10^{-9}	5.9×10^{-9}	2.1×10^{-8}	6.4×10^{-8}			
2D zinterol	2.0×10^{-8}	5.0×10^{-9}	1.5×10^{-8}	5.0×10^{-8}	1.5×10^{-7}	5.0×10^{-7}	1.5×10^{-6}		0.20
propranolol	6.5×10^{-10}	0	1.3×10^{-9}	5.9×10^{-9}	1.9×10^{-8}	6.5×10^{-8}	2.0×10^{-7}		
3A isoproterenol	3.0×10^{-7}	1.0×10^{-8}	3.0×10^{-8}	3.0×10^{-7}	3.0×10^{-6}				0.032
metoprolol	2.4×10^{-7}	0	4.8×10^{-7}	7.0×10^{-6}	7.2×10^{-5}				
3B zinterol	2.0×10^{-8}	1.0×10^{-8}	3.0×10^{-8}	1.0×10^{-7}	3.0×10^{-7}	1.0×10^{-6}	3.0×10^{-6}		0.33
metoprolol	2.4×10^{-7}	0	4.8×10^{-7}	2.2×10^{-6}	7.0×10^{-6}	2.4×10^{-5}	7.2×10^{-5}		

the concentration of bound receptors progressively changes from the condition represented in Fig. 4A to that in Fig. 4B. It is apparent that under the conditions in Fig. 4B the mobility of the receptors will determine the maximum cyclase activation rate; the results indicate that the mobility of receptors is insufficient to account for the extent of activation observed for isoproterenol stimulation in the absence of any antagonist. In contrast, the comparatively high binding frequency of metoprolol precludes such an occurrence, as depicted in Fig. 4C.

Figs. 5–7 show simulations of receptor binding for the metaproterenol, dobutamine, and zinterol experiments. Zinterol is a special case in these experiments, because it has a relatively high affinity (K_d = 20 nM) that is presumably reflected in a long half-life of the bound state (as has been assumed in the simulation). Thus, propranolol occupancy can probably not alter receptor participation with zinterol on a small time scale. This is analogous to the use of metoprolol in the antagonism of isoproterenol, wherein the apparent binding frequencies of agonist and antagonist are relatively closely matched and the presence of one under this protocol cannot significantly affect the distribution of receptor occupancy of the other. It is important to note that the precise values for the dissociation constants used in the simulations shown in Figs. 4–7 are not crucial to the demonstration of the effects on occupancy of the experimental conditions; rather, the important factor is that the dissociation rates for isoproterenol, metaproterenol, dobutamine, and metoprolol are rapid, compared with that for propranolol. More important, the basic conclusion that binding frequency contributes to the PSR for isoproterenol does not rely on precise knowledge of the K_d values for either agonist or for the antagonist propranolol (discussed in Ref. 4); the experiment is designed to have a fixed concentration of agonist-bound receptors, but the comparison of one response point with the control point does not require a specific value for that concentration in order to be valid.

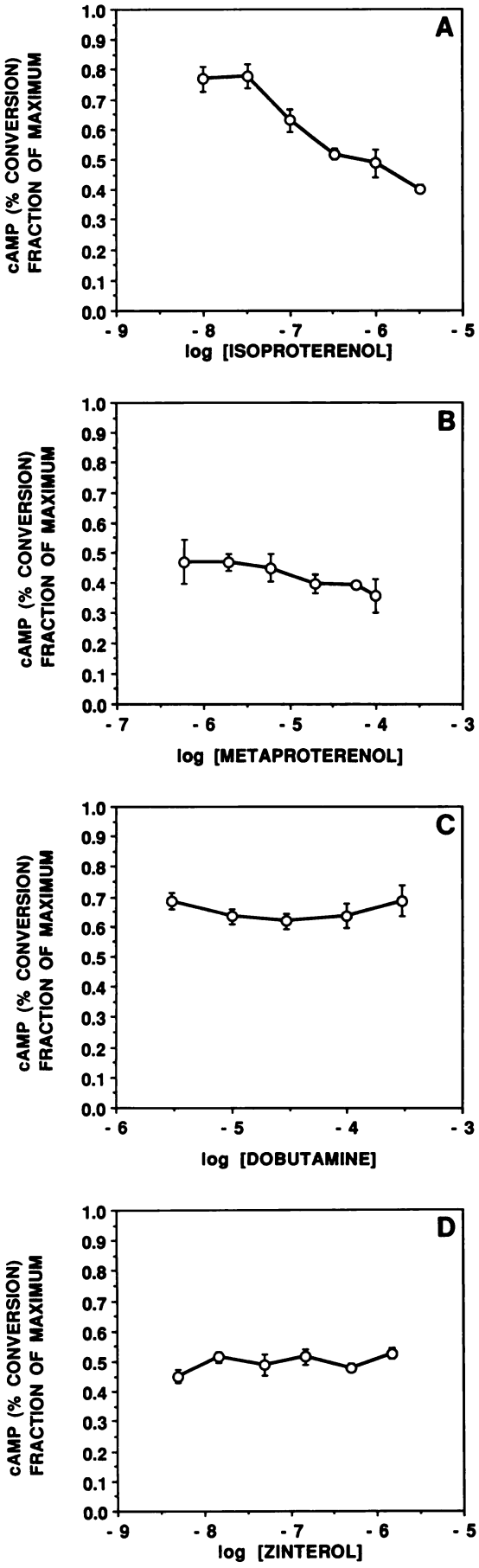
Discussion

The separation between the binding curve (characterized by the K_d) and the activation curve (characterized by the EC_{50}) is a distinguishing property for a β-adrenergic agonist (characterized by PSR = K_d/EC_{50}). The collision coupling model of Tolkovsky and Levitzki (1) can accurately characterize the relationship between the binding and response curves for any known β-adrenergic receptor agonist, with the assumption that the rate of activation of G protein/cyclase is proportional to the concentration of agonist-bound receptors (2, 12).

For epinephrine, it has previously been shown that there are circumstances for which the assumption of the proportionality between bound receptor concentration and the cyclase activation rate leads, however, to incorrect predictions (4). Those circumstances relate to the way in which the bound receptor concentration is obtained, whether by fractional occupancy of the entire receptor population (the normal case for occupancy turnover) or by some greater occupancy (occupancy for a greater fraction of the time) of a smaller fraction of the total number of receptors (restricted occupancy turnover). Using the antagonist propranolol, we can force a reduction in the turnover of occupancy, such that the same receptor-agonist complex concentration [AR] is obtained by a reduced absolute number of receptors. In the experiments reported above, this approach was used to examine potential contributions of the turnover of agonist occupancy to cyclase activation for a series of β-adrenergic agonists that cover the available range of PSRs in the S49 cell.

For isoproterenol, the experiment showed a reduction in cyclase activity, compared with control (a point on the normal dose-response curve), when occupancy turnover was restricted. For metaproterenol, the cyclase activity under such conditions was marginally less than control, whereas for zinterol and dobutamine no effects of the restriction of occupancy turnover were observed.

The results presented here and previously (4) have demonstrated a reduction in cyclase activation for certain agonists under circumstances in which such changes are not anticipated by collision coupling for any agonist. These results can be explained by a consideration of one aspect of the interaction between β-adrenergic receptors and G protein that is only slightly modified from the original collision coupling model. The difference is shown schematically in Fig. 8. In the original model, a collision between a receptor and G protein takes place once, with a receptor that is strictly either bound or unbound, and thus the rate of activation of G protein is proportional to receptor occupancy. The modified scheme includes the concept that receptors and G protein probably collide a number of times in sequence (what we call an encounter) over some short period of time (4). An explanation for the difference in cyclase activation depending on the turnover of receptor occupancy, using the modified scheme, is possible and straightforward if the mean duration of this series of collisions is greater than the mean lifetime of receptor-agonist complexes for some agonists. If an agonist is efficient enough to activate G within a fraction of the apparent period of an encounter between a receptor and



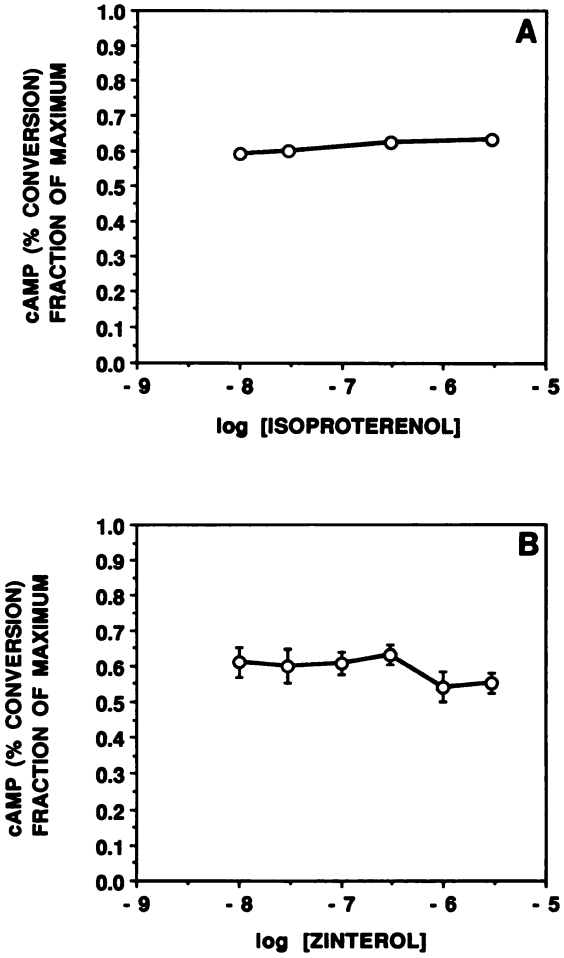
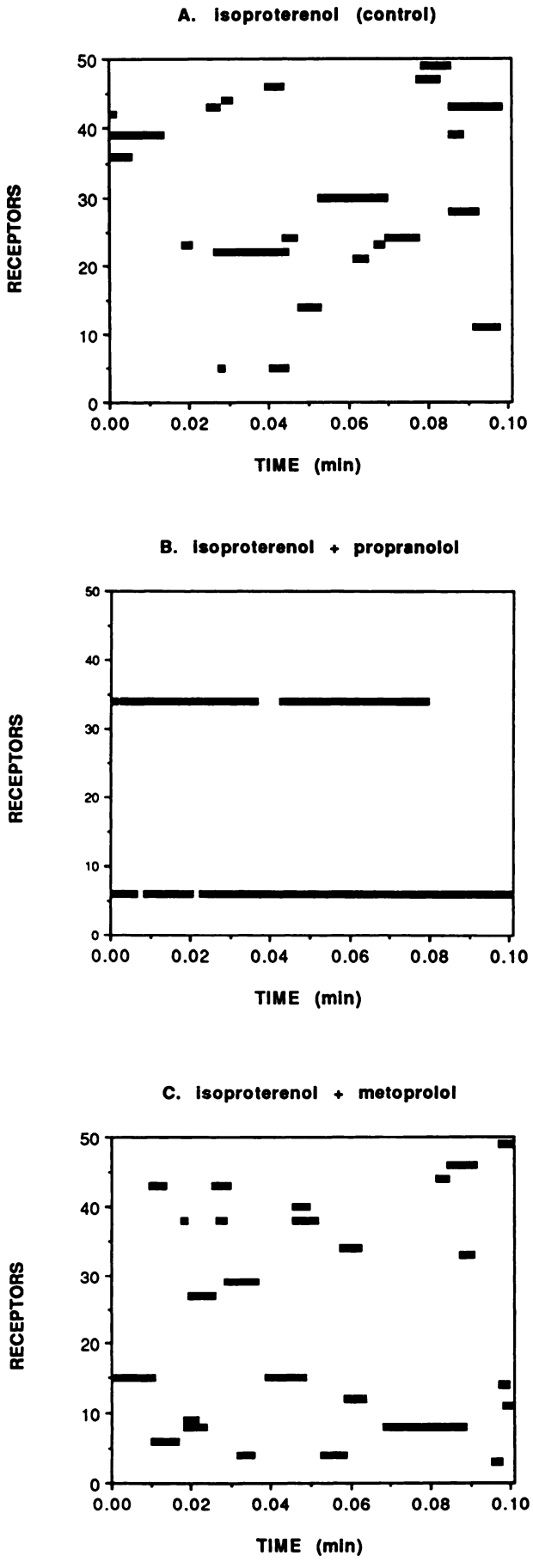


Fig. 3. cAMP accumulation (1-min percentage of conversion, average of triplicates ± standard error) stimulated by agonist in the presence of metoprolol with a constant concentration of agonist-bound receptors. As in Fig. 2, both agonist and antagonist concentrations are increasing from *left* to *right*, although only the agonist concentrations have been labeled on the plots. The corresponding metoprolol concentrations are given in Table 2. A, Isoproterenol; B, zinterol.

G protein, then rapid dissociation of agonist (high binding frequency, where at steady state the occupancy is statistically maintained by association of agonist at some other receptor) enables occupancy to be continuously distributed away from any receptor for which further collisions in the same sequence of collisions with a G protein would be wasted. This results in a component of the PSR for an agonist that is attributable to binding frequency, when the agonist is also very efficient, per

Fig. 2. cAMP accumulation (1-min percentage of conversion, average of triplicates ± standard error) stimulated by agonist in the presence of propranolol, with a constant concentration of agonist-bound receptors. In each plot, both agonist and antagonist concentrations are increasing from *left* to *right*, although only the agonist concentrations are shown. The corresponding propranolol concentrations are given in Table 2. For each *point* in a given plot, the concentrations of agonist and antagonist were calculated to give the same concentration of agonist-bound receptors at steady state binding, and split additions of propranolol before and concurrent with agonist addition were used to obtain a rapid approach to that steady state. Each plot is representative of at least two experiments. A, Isoproterenol; B, metaproterenol; C, dobutamine; D, zinterol.



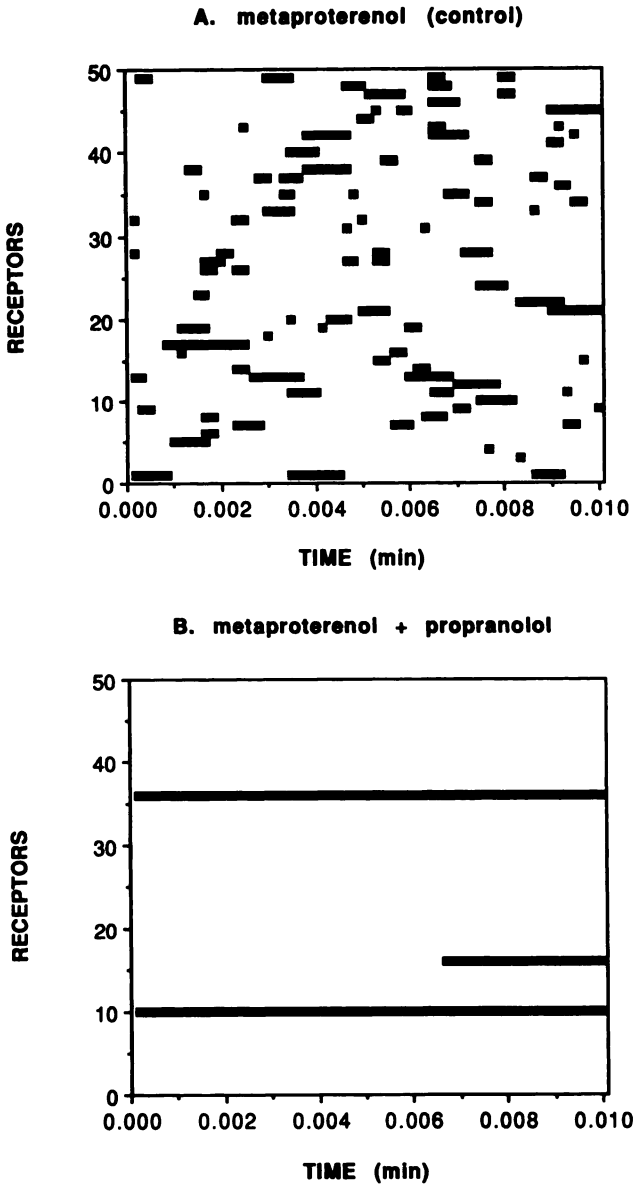


Fig. 5. Simulations of individual receptor binding to metaproterenol at steady state, corresponding to conditions in Fig. 2B. A, 600 nM metaproterenol (control); B, 100 μM metaproterenol, 100 nM propranolol.

collision, at activating G protein. Because of this, there is one particular circumstance (high occupancy of a low number of receptors by an efficient, high binding frequency agonist) for which the collision coupling model is insufficiently detailed to

Fig. 4. Simulations of individual receptor binding to isoproterenol at steady state, corresponding to conditions shown in Figs. 2A and 3A. A, 10 nM isoproterenol (control); B, 3 μM isoproterenol, 240 nM propranolol; C, 3 μM isoproterenol, 72 μM metoprolol. The binding state of an individual receptor is represented by the color (*black* or *white*) within a horizontal track in the figure (80 tracks total, numbered along the *y*-axis and parallel to the *x*-axis) moving through time (the *x*-axis, Δt = 0.1 min) from *left* to *right*; *black*, bound to agonist; *white*, unbound or bound to antagonist. The width of the tracks (and the corresponding overall height of each figure) is arbitrary, but the tracks were plotted exactly adjacent to each other, such that the fraction of the area of the plot that is black is proportional to the fractional occupancy by agonist of the receptors under these conditions, in order to demonstrate different ways in which the same fractional occupancy can theoretically be achieved. For a sufficiently large number of simulations, as in A, B, and C, the average

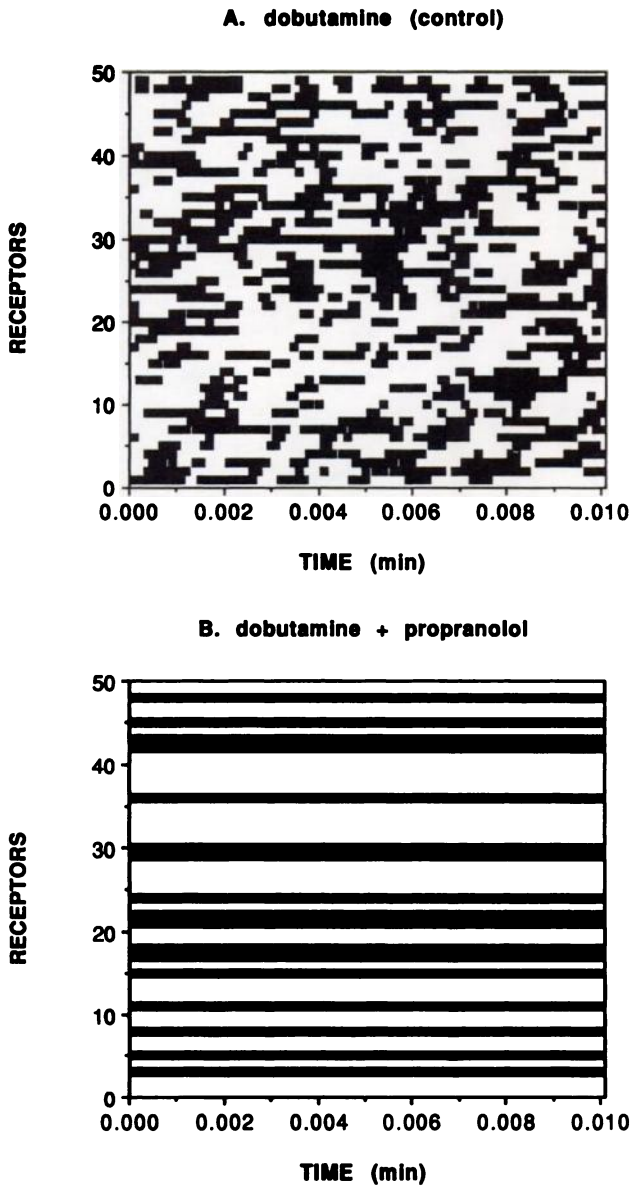


Fig. 6. Simulations of individual receptor binding to dobutamine at steady state, corresponding to conditions in Fig. 2C. A, 3 μM dobutamine (control); B, 300 μM dobutamine, 64 nM propranolol.

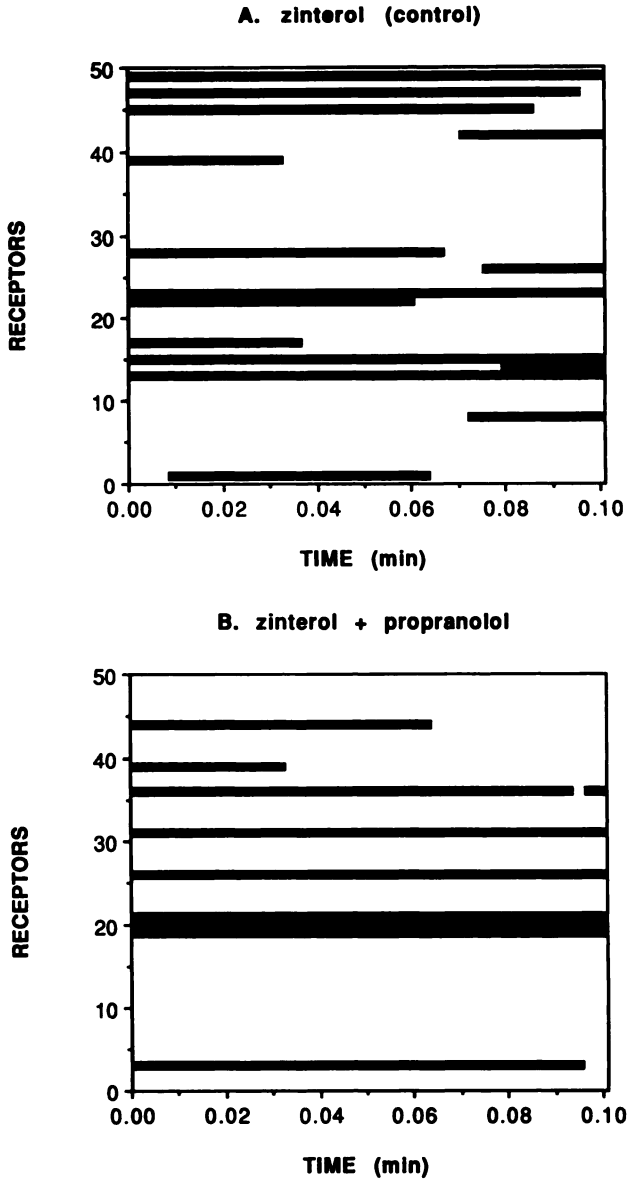


Fig. 7. Simulations of individual receptor binding to zinterol at steady state, corresponding to conditions in Fig. 2D. A, 5 nM zinterol (control); B, 1.5 μM zinterol, 200 nM propranolol.

predict the observed results. The fundamental aspect of the collision coupling model, in which agonist-bound receptors act as mobile catalysts for the activation of G protein, remains intact. The difference in the encounter model is that collisions of receptors with G protein occur in packets (an encounter), and each occurrence of a series of collisions involves a finite time.

According to this interpretation, there are two agonist-specific factors that influence the PSR and that are independent. First, the binding frequency of the agonist (analogous to a "turnover" number for the interaction between individual receptors and agonist) determines the number of individual receptors involved in the process of encounter coupling per unit time. Second, the efficiency of the receptor at activating G protein while the receptor is in the bound state is particular to an agonist. If we make the assumption that agonist affinity is a reflection of the half-life of the receptor-agonist complex (i.e., that a relatively low affinity agonist has a relatively short agonist-receptor half-life), then the agonists we have used here are examples of four different combinations of these variables, in agonists that cover the range of observed PSRs, (a) high binding frequency, high efficiency (isoproterenol), (b) high binding frequency, low efficiency (dobutamine), (c) low binding

occupancy by agonist under each condition is identical. The simulations were carried out as follows. For each track, the initial condition was set according to the probability of being bound to agonist (the agonist occupancy) (θ), given the concentrations of agonist and antagonist and the K_d values for each. In each simulation step, the probability of changing from one state to another (i.e., from bound to unbound, from unbound to [bound to agonist], or from unbound to [bound to antagonist]) was a function of the simulation step size dt, the concentrations of agonist and antagonist, and the rate constants for association and dissociation of agonist and antagonist, according to standard reaction kinetics. A change in state was made according to the calculated probabilities of change, which were compared with the output (0–1) from a random number generator at each step.

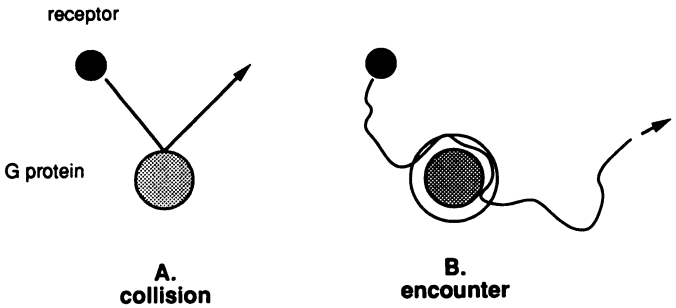


Fig. 8. Schematic illustrations of collision coupling model (A) and encounter coupling model (B). In the collision model, the interaction between receptor and G occurs with a receptor that is either bound or unbound, with the probability that any particular collision occurs with a bound receptor being equal to the overall receptor occupancy by agonist. In the encounter model, the interaction between receptor and G involves a series of collisions that has some duration, which can be longer than the mean lifetime of the receptor-agonist complex for some agonists. In the collision model, the rate of G activation is proportional to receptor occupancy, which is proportional to the concentration of agonist-bound receptors. In the encounter model, the distribution of occupancy time affects the relationship between the concentration of agonist-bound receptors and the rate of G activation.

frequency, low efficiency (zinterol), and (d) high binding frequency, intermediate efficiency (metaproterenol). The combined contribution of efficiency and binding frequency to the dose-response curve was examined by an experimental approach that attempts to compare cyclase activity at a point on the mobility-limited dose-response curve with that at a point on the normal dose-response curve, each in response to the same concentration of agonist-bound receptors. The mobility-limited dose-response curve is one in which the concentration of bound receptors is composed of 100% occupancy of a variable (fractional) number of receptors, in contrast to the normal dose-response curve, in which the concentration of bound receptors is given by fractional occupancy of the normal number of receptors. For reasons discussed below, the experimental approach can only approximate the mobility-limited case. However, it is clear from the experiments that the two circumstances can be quite different with respect to the observed rate of adenylate cyclase activation, but only for a high binding frequency, high efficiency agonist such as isoproterenol.

For agonists where a reduction in cyclase activation can be observed in experiments such as those reported here, the reduction may be viewed as being equivalent to an overall increase in the EC_{50} and a corresponding decrease in PSR (4). Thus, high binding frequency, high efficiency agonists such as isoproterenol and epinephrine have a normal dose-response curve that is shifted leftward from the mobility-limited dose-response curve. In other words, there is a fraction of the PSR for these agonists that is attributable to the time distribution of occupancy among the receptor population, and for these agonists the rate of activation of cyclase is greater for small occupancy of a large number of receptors than for high occupancy of a small number of receptors. When this is the case, there is a maximum extent to which the PSR is attributable to receptor mobility, which should be independent of the agonist because mobility is probably largely independent of occupancy. The experiments measure a shift of the normal dose-response curve to the mobility-limited dose-response curve (a rightward shift). What appears to be very similar, if not identical, in the mobility-limited dose-response curves for epinephrine and isoproterenol is the extent that they are separated from the receptor binding curve (a leftward shift). For epinephrine, we previously measured a 6-fold shift to the right of the dose-response curve, leaving approximately a 30-fold separation from the epinephrine binding curve. For isoproterenol, we measured a decrease in the response for the mobility-limited case that corresponds to an increase in the EC_{50} from 2–3 nM to 5–6 nM, or a 3-fold shift of the dose-response curve to the right. This leaves approximately a 30-fold separation between the mobility-limited isoproterenol dose-response curve and the isoproterenol binding curve, which is the same separation measured previously for epinephrine. It is important to note that, in the context of such a model for encounter coupling, then not only would we predict a 30-fold separated mobility-limited dose-response curve, but correspondingly we would predict that the maximum EC_{50} increase that we could observe would be, at most, a factor of <4 for a normal dose-response curve that has a PSR of approximately 100. For these same reasons, it is expected that the observed decrease in cyclase activity in the mobility-limited case would be only barely observable for metaproterenol, which has a PSR of 40. Thus, the contribution of metaproterenol binding frequency to its PSR, although it probably exists, is only a small factor.

For agonists such as dobutamine and zinterol, the normal PSR is not as great as the mobility-limited PSR. There are at least two factors that, independently or in combination with each other, can cause this circumstance. First and most obviously, the intrinsic efficiency of the receptor in the bound state to activate adenylate cyclase may be low. Thus, if the probability of activating cyclase in any given encounter is low, there is no advantage to "switching" occupancy to another receptor (possibly another, different, encounter). Dobutamine appears to be an example of this. Second, it may be the case that binding frequency is very low, a case in which switching does not take place on a time scale that is less than the encounter time and thus renders no advantage to the cyclase activation rate. Zinterol appears to be an example of this. Metaproterenol is an intermediate case, wherein binding frequency and apparent activating efficiency are sufficient to give a PSR that is marginally greater than the mobility-limited PSR.

In our interpretation of the data, we have assumed a single population of receptors whose gross physical parameters are not affected by agonist binding, i.e., receptor mobility does not alter with agonist occupancy and there is no high affinity component to agonist binding in intact cells. Our precise assumption with respect to receptor mobility is that receptors spend a finite (but variable in a statistical sense) time in proximity to or in a succession of collisions with G_s/cyclase. Only in some very refined quantitative senses (which we are not attempting here) would it matter if the receptor mobility varied with agonist occupancy, and indeed mobility could be variable without affecting our conclusions in any significant way. With respect to binding affinities, we have never been able to detect experimentally a high affinity component of agonist binding in our preparations of intact S49 cells, which means that it must account for only a small percentage of the total binding. It has been shown that in the presence of GTP the high affinity ternary complex (hormone-receptor-G_s/C) is very short-lived in membrane preparations (8) and contributes

little to hormone binding. It is reasonable to hold that the same situation applies in intact cells.

The potential effects of receptor desensitization and of PDE activity on the results are probably small. Although significant desensitization of receptors in S49 cells during a 1-min incubation occurs with all of the agonists, its effect on the cAMP accumulation during 1 min is quite small, as is evidenced by the near-linearity of accumulation during that minute (13). This may be in part because of the relatively long activation time for the cyclase (in the Cassel-Selinger scheme), which will cause a lag in the desensitization measured by cyclase activity relative to that measured by receptor activity. We have made extensive measurements on the level of PDE activity in intact S49 cells (14). Typically, in these cells the fractional turnover constant for cAMP is in the region of 0.1 min^{-1}. The fraction of synthesized cAMP that is hydrolyzed in the first minute after the onset of stimulation is, therefore, rather small. Although we believe that the effects of PDE activity and of desensitization on our data are small, at the key level of interpretation of the data it would not matter if those effects were quite large. The essence of the experiments is to show that at constant receptor-agonist concentration there is less response for some agonists when they are forced to occupy a smaller number of receptors for a greater fraction of the time. If this phenomenon were not real it would not be observed, irrespective of the effects of desensitization or hydrolysis on the 1-min accumulation.

In our interpretation of these data, we have attributed the differences in K_d values among the agonists to differences in their rates of dissociation, and we have made the corresponding assumption that the higher affinity agonists have longer agonist-receptor lifetimes. More precisely, we have assumed that the association and dissociation rates of the agonists are rapid, compared with that for propranolol. It has been shown that equilibration of isoproterenol binding is achieved within seconds (9, 15), but figures for direct measurements of the rate constants are not available, because the rate constants for association and dissociation for all of the agonists used here are too rapid to be measured by conventional means. It is important to note, however, that the design of the experiments is not dependent in any way on assumptions about the kinetic rate constants but is based on their measurable ratios (the dissociation constants, K_d). We have assumed in the simulations that the rate constants for association of the agonists are all equal to that for propranolol (4×10^8/M/min), which number can be calculated from its measurable K_d and its measurable (because it is slow) rate of dissociation. A similar value for the association rate constant of isoproterenol to β-adrenergic receptors on polymorphonuclear leukocytes, $>10^8$/M/min, has been derived from an analysis of the kinetics of the functional effects of isoproterenol on those cells (16).

The use of propranolol to limit the switching of agonist receptor occupancy among the entire receptor population can only approximate the ideal case in which receptor occupancy is completely isolated to a fixed number of receptors, although the approach is sufficient to reveal the effects of occupancy turnover (or switching) on cyclase activation. By design, the effective half-life of an agonist-receptor complex is increased in the experiments by making it most probable that the dissociation of agonist from an unbound receptor is rapidly followed by the association of another agonist molecule. The high probability of rapid association of agonist is likewise true for any receptor that is unbound, and thus an unbound receptor from which propranolol has dissociated is equally likely to become rapidly bound to agonist. Because of the long mean lifetime of the propranolol-receptor complex (t_m = 230 sec), the majority (approximately 75%) of the receptors that are initially bound to propranolol do not undergo such an exchange during the 60-sec duration of the experiment. However, approximately 25% of the receptors that are initially bound to propranolol lose propranolol during that time and are likely to gain agonist. (Because the binding is at steady state, the same number of different receptors lose agonist and gain propranolol.) Thus, it is probable that a minimum of this amount of switching of occupancy among the receptor population occurs. Nonetheless, a significant decrease in cyclase activation can be observed for some agonists in such an experiment and, although the extent of decrease appears to approach a limit, presumably there could be an even greater decrease in activation if the desired complete isolation of occupancy to a fixed number of receptors were achieved (e.g., by using an antagonist with a longer bound half-life or by using an irreversible receptor blocker with an infinite half-life). The approach used here is a very practical one, however, because it can be enacted rapidly and with reproducible precision (4). Note, again, that the closest approach to the ideal case (one in which receptors would be completely removed from participation with agonist) is the rightmost point in Figs. 2 and 3. The results of greatest interest are those rightmost points, whereas the other points were measured in order to map the transition in cyclase activity from control (no antagonist) when such changes occurred.

On the basis of these results, we claim that the PSR (K_d/EC_{50}) can be understood in terms of both an intrinsic efficiency for cyclase activation and a potential component due to binding frequency and occupancy turnover. The effect of binding frequency is observable when the efficiency is apparently high and when the half-life of the agonist receptor complex is short. Assuming that association rate constants are similar for all these agonists, then an agonist with a short receptor half-life may be said to have (in relative terms) a high binding frequency or a high occupancy turnover (i.e., a given overall occupancy involves a large number of receptors per unit time). Our interpretation of the results we have obtained leads to the prediction that, for a given efficiency, an agonist with a higher affinity should have a lesser PSR, because a higher affinity is likely a result of a longer bound lifetime. There is other evidence to support this interpretation. In a series of papers, Melmon and co-workers (Refs. 17 and 18 and associated references) described the synthesis of numerous catecholamine derivatives and an evaluation of their effects on cyclase activation in intact cells and their binding to membrane preparations. For some of those compounds, both an increase in receptor affinity and an increase in potency (in a standard pharmacological sense of relative concentration required to elicit a specified degree of effect) were achieved, but in no cases was there an increase in the ratio of binding to effect (equivalent to the K_d/EC_{50} ratio); it was decreased. Our interpretation of the results presented here and earlier can explain this relationship. According to our interpretation, it is because the higher affinity is due to a longer half-life of the receptor-agonist complex, with the concomitant loss of whatever advantage a more rapid turnover of occupancy could lend to the rate of activation per degree of occupancy

and, hence, to the level of activation at any degree of occupancy. If a "better" agonist is defined as one that exhibits an increased affinity and an increased PSR, it is probable that in this system a better agonist cannot be obtained beyond some limit. For instance, zinterol, compared with isoproterenol, has a higher affinity, is equally potent, and possess full efficacy, but zinterol has a lower PSR than isoproterenol. There are, to our knowledge, no examples of an agonist with both a greater affinity and a greater PSR than isoproterenol.

There is potential here for an incorrect analogy to be drawn between the rate theory of Paton (19) and the concepts presented here. Paton's rate theory was based on the idea that an agonist transmitted its action upon association with receptor, with the rate of activation equal to the rate of association (or, equivalently at steady state, dissociation). Here the receptor is active only in its bound form and remains active while in its bound form, with no conflict with the normal current understanding of receptor activity. The net rate of G protein/cyclase activation can, however, depend on the rate of turnover of occupancy among the receptor population, where a short lifetime of the agonist-bound state, equivalent to or characterized by a high rate of dissociation of agonist, can increase cyclase activation per degree of receptor occupancy. The dependence is not due in any way to the act of association or dissociation of agonist from the receptor but, rather, is due to the fact that the release of agonist from one receptor is statistically replaced by binding of another receptor in some different location on the cell surface. The net effect of rapid dissociation may be thought of as a mobility of receptors enhanced by binding frequency; the "jumping" of occupancy to another receptor increases the likelihood that another G protein will be encountered. If an agonist-bound receptor is efficient enough to activate G during one cycle of binding and unbinding that occurs within an encounter, then the overall rate of G activation will be partly dependent on the rate at which this turnover of occupancy among the entire receptor population occurs. This dependence would be nil if an encounter had zero duration, as in a single collision, because the probability of a collision with a bound receptor would be proportional to occupancy (i.e., it would occur with a receptor that is either bound or unbound, with no change in state during a zero-duration collision), no matter how rapid was the turnover of occupancy with respect to individual receptors. Similarly, there will be no significant contribution of occupancy turnover to the activation rate when the lifetime of a receptor-agonist complex is so long that a change in state of an individual receptor during an encounter is unlikely or if the efficiency of the agonist is so low that the probability of activation in any encounter is low.

We have demonstrated that, for two agonists (epinephrine and isoproterenol), the time-dependent distribution of occupancy is a factor that adds to the extent of activation per degree of occupancy. According to our interpretation of these results, for these agonists there is an "advantage" to having a low affinity for its receptor, in the sense that a shorter half-life of the agonist-receptor complex (reflected in a higher K_d) is a factor that enhances the cyclase activation rate per occupied receptor and that contributes to the high PSRs observed for these two agonists in this system. This result is somewhat counterintuitive but is a consequence of a combination of two conditions; (a) the duration of an encounter between receptors and G protein is longer than the half-life of either of these agonist-receptor complexes and (b) the agonist-receptor complex for these agonists is apparently highly efficient at activation of G protein/cyclase. The effect of decreased activation rate for the mobility-limited case would not occur if either of these conditions were not met. According to this analysis, agonist binding frequency and efficiency are thus coupled variables in the relationship between binding and response in β-adrenergic agonism.

There is some difficulty in assessing without speculation the pure physiological relevance of this phenomenon, even in a teleological sense (i.e., to explain why epinephrine, a naturally occurring agonist, might usefully have the property that its binding frequency would have some impact on its net induced activity). However, potential consequences of the phenomenon are relevant to both clinical and basic pharmacology, particularly with respect to the activity of agonists in the presence of antagonists. From a clinical standpoint, we have cited the potential effect of propranolol to act as a "frequency filter" for adrenergic activity, at least in comparison with a lower affinity antagonist such as metaprolol (4). From a pharmacological standpoint, the results demonstrate that the effective inhibition constant for an antagonist, which at the molecular level is derived simply from competitive occupancy of the receptor, nonetheless is not constant in this system but depends on the relative rates of turnover of both agonist and antagonist with respect to individual receptors. In this system, propranolol can be a more potent antagonist than one would predict on the basis of competition (i.e., its inhibition constant, K_i, is less than its dissociation constant, K_d, in the Cheng-Prusoff relation described above) under circumstances equivalent to ones in which receptor number is reduced. Thus, there are circumstances in which, in the presence of a given agonist, the inhibition constant of an antagonist can "slide" away from its K_d. Another perspective on the relevance of the phenomenom is simply that of a better quantitative understanding of one aspect of a signal transduction pathway involving coupling of receptors to G proteins. Clearly, the mechanism and quantitative rules governing the relationship between receptor occupancy and activity need to be well defined and understood if the mechanisms underlying feedback receptor regulation and desensitization are to be understood.

These results further demonstrate certain details of the relationships between agonist, receptor, and G protein, which involve factors corresponding to an apparent encounter time for receptor-G protein interaction, agonist efficiency, and the agonist-receptor complex half-life. There exists a component of the PSR that is due to binding frequency only when the mean lifetime of the receptor-agonist complex is less than the duration of an encounter between receptor and cyclase and when the intrinsic efficiency of the agonist for G protein activation is high.

Acknowledgments

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