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# Mechanism of Activation of $A_2$ Adenosine Receptors. II. A Restricted Collision-Coupling Model of Receptor-Effector Interaction

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**SUMMARY**

Existing models describing the kinetics of receptor-effector interaction were found to be insufficient to account for the experimental findings on adenylate cyclase activation by $A_2$ adenosine receptors described in the preceding manuscript [*Mol. Pharmacol.* **39:** 517–523 (1991)]. We have, therefore, chosen another approach and have developed discrete computer simulations of receptor-effector interactions taking place on a spherical membrane. These simulations were based on the following principles: (a) receptors activate effectors in a catalytic manner, and (b) diffusion of receptors and effectors is slow, so that receptors will only activate effectors that are in their vicinity at the time of agonist occupation. Using several experimentally determined parameters, these simulations could reproduce the experimental findings on adenylate cyclase activation by $A_2$ adenosine receptors described in the preceding manuscript. In addition, by appropriate choice of the simulation parameters, they are shown to accommodate the behavior of several other models of receptor-effector interactions.

The regulation of adenylate cyclase activity by hormone receptors has been studied by many authors as a prototypical transmembrane signaling system, in order to develop mathematical models of receptor-effector interactions (see reviews in Refs. 1–3). Although the chain of events leading from the occupation of a receptor by an agonist to the stimulation (or inhibition) of adenylate cyclase is a complex event involving activation and probably dissociation of the $G_s$ (or $G_i$) protein (4), it has been observed repeatedly that the process can be adequately described in terms of a bimolecular reaction between receptor and effector (3). In particular, the time course of adenylate cyclase activation by hormones is, in general, first order, i.e., follows a monoexponential curve. Thus, $G_s$ and the catalytic subunit of adenylate cyclase can be regarded in kinetic terms as a single effector unit. Likely mechanistic explanations for this simple behavior are a tight association between $G_s$ and the catalytic subunit, either in physical or in kinetic terms, or a large excess of $G_s$ over receptors and catalytic subunits (2, 5). Based on the simple kinetics of adenylate cyclase activation, two simple possible models have been proposed by Levitzki and co-workers (2, 3, 6–8). The "precoupled model" assumes fixed coupling between receptors and effectors, whereas the "collision-coupling" model assumes a catalytic activation of effectors by receptors.

Both models make strict predictions about the effects of reduction of the receptor number on the rate and extent of irreversible effector activation (i.e., in the presence of nonhydrolyzable GTP analogues in the case of adenylate cyclase activation); the precoupled model predicts a proportional reduction of the extent but no effect on the rate of activation, whereas the collision-coupling model predicts a reduction of the rate but not the extent of activation (6). Using this experimental approach, the $\beta$-adrenergic receptor of turkey erythrocyte membranes, as well as that of rat caudate membranes, has been reported to activate adenylate cyclase according to the precoupled model, whereas the $A_2$ adenosine receptors of the same membranes have been reported to activate adenylate cyclase according to the collision-coupling model (6–9). Although some of the experiments on which these theories were based have been questioned as possibly being due to P site-mediated inhibition by adenosine (10), the kinetic as well as the theoretical arguments still appear valid.

However, in the preceding manuscript, we observed that a reduction of the number of $A_2$ adenosine receptors in human platelet membranes led to a less than proportional decrease of both the extent and the rate of adenylate cyclase activation. Because this observation is compatible with neither of the simple models mentioned above, it calls for new approaches for dealing with this question.

**ABBREVIATIONS:** NECA, 5′-*N*-ethylcarboxamidoadenosine; Gpp(NH)p, guanosine 5′-($\beta$,$\gamma$-imido)triphosphate; (*R*)-AHPIA, (*R*)-2-azido-$N^6$-*p*-hydroxyphenylisopropyladenosine.

An obvious problem with models assuming catalytic activation of effectors by receptors, such as the collision-coupling model of Levitzki and co-workers (2) or the allozyme hypothesis of Macfarlane (11), lies in the fact that they use equations derived from enzymology to describe not only the interaction between agonists and receptors but also that between receptors and effectors. However, the mobility of receptors in the plasma membrane is low, compared with the speed with which effector activation occurs (12). Therefore, it is unlikely that the agonist-occupied receptors have free access to their substrates, the effectors, in the same manner as in an enzymatic reaction taking place in solution. It appears rather more likely that receptors have access only to those effectors that are in a restricted area in their vicinity, even when prolonged time intervals are observed. A model that is based on a structural assembly of receptors and effectors in two matrices, and that introduces such a restricted interaction field, has been proposed by Bergman and Hechter (13). However, this model arranges receptor and effectors in quasicrystalline arrays, which is unlikely to be a true reflection of the arrangement of membrane proteins and which may result in the generation of artifacts.

We have, therefore, developed a model allowing simple computer simulations of receptor-effector interactions, based on a random distribution of receptors and effectors and on movement by diffusion within the layer of the plasma membrane. We show that these simulations accommodate our experimental results as well as the predictions of a number of models developed by others.

## The Model

The simulation model of restricted collision-coupling has the following general properties. (a) Receptors and effectors are randomly distributed on a spherical membrane. They move in a random manner according to the laws of diffusion. (b) A single agonist-occupied receptor can sequentially activate several effectors during the individual lifetime of the agonist-receptor complex. For the sake of simplicity, it is assumed that activation of an effector occurs each time an agonist-receptor complex and an inactive effector collide. Receptors can be in the states "free," "agonist-occupied," or "blocked," and effectors can be in the states "free," "activated," or "blocked." Blocked receptors or effectors are disregarded in the simulation process; this allows the simulation of different stoichiometries. For our simulations, 3000 receptors and 3000 effectors were randomly distributed on a sphere (but any numbers can be used to accommodate computing limitations).

It is assumed that dissociation of agonist-receptor complexes occurs exponentially, with a mean lifetime of $T_R$, and, likewise, that activated effectors return to an inactive state with a mean lifetime of $T_E$ (see Table 1 for an explanation of simulation variables). For an individual agonist-receptor complex, an individual time of occupancy, $t_R$, is calculated using a random number generator for exponential distribution, with the parameter $\tau_R = 1/T_R$. Similarly, for an individual activated effector, an individual active time, $t_E$, is calculated using a random number generator for exponential distribution, with the parameter $\tau_E = 1/T_E$.

The time between occupation of a given receptor by agonist and activation of a given effector by this agonist-receptor complex is called $t_a$. This time is dependent on the distance between the agonist-receptor complex and the effector and on the diffusion parameter. We used the first-passage time distribution of the one-dimensional Brownian motion applied to the distance between the agonist-receptor complex and the effector to produce a random variate for $t_a$. Distances were calculated according to spherical trigonometry. The diffusion parameters were varied. Individual $t_a$ times were calculated for all effectors in a circular area around an agonist-receptor complex, containing 10 ± 4 effectors (mean and standard deviation). This area constitutes the vicinity or "interaction field" (13) of a receptor. An effector will only be activated by an agonist-receptor complex if the calculated $t_a$ is smaller than the calculated $t_R$, i.e., if the receptor is still agonist occupied at the time of collision with effector. Collisions of an agonist-receptor complex with already active effector are disregarded.

TABLE 1
**Simulation variables**

| Variable | Definition |
|---|---|
| $N_{hit}$ | Number of hits (=agonist-receptor collisions)/min |
| $t_R$ | Individual lifetime of agonist-receptor complex (exponentially distributed, with mean $T_R$ for all agonist-receptor complexes) |
| $T_R$ | Mean value of $t_R$ |
| $t_E$ | Individual lifetime of activated effector (exponentially distributed, with mean $T_E$ for all effectors) |
| $T_E$ | Mean value of $t_E$ |
| $t_a$ | Time between agonist occupation of an individual receptor and subsequent activation of an individual effector |
| $D$ | Diffusion parameter |

Uniform pseudorandom numbers were generated using a long-period congruential random number generator (14, 15). Different seeds create independent sequences of such numbers. These sequences form the base for all other random variates. Random variates for the normal and exponential distributions were generated as described by Knuth (16). The random distribution of receptor and effector on the sphere was achieved using the method of Knop (17). Random variates for the first-passage time distribution (18) were generated as outlined by Devroye (19).

The simulation process runs on two arrays, containing information about all 3000 receptors and effectors, respectively. Initially, no receptors are occupied and no effectors are activated. In order to simulate different stoichiometries of receptor and effector, a randomly distributed percentage of receptors and/or effectors may be blocked.

All events in the simulation process take place at constant ticks of a simulation clock. The number of ticks/min reflects the agonist concentration. At each tick, one agonist molecule collides in a random manner with a receptor; this is called a hit. The collision leads to occupation of the receptor by the agonist, if the receptor is not already occupied. At the time of occupation, the lifetime of the agonist-receptor complex ($t_R$) and the delay times ($t_a$) after which the different effectors in the interaction field of the receptor will be activated are calculated using random number generators, as described above. At the time of activation of an effector, an individual lifetime for this activation is calculated as described above.

A flow chart of the main loop of the simulation process is shown in Fig. 1. Details of the simulation and the calculations are given in the Appendix.

This general scheme can be adapted to many different situations by varying the relevant simulation parameters. (a) If agonist occupation of the receptors is irreversible, the mean occupation time of receptors, $T_R$, can be set to a high value. (b) If activation of effectors is irreversible, the mean lifetime of activated effectors, $T_E$, can be set to a high value. (c) Mean lifetimes of receptor occupancy and effector activation can be adjusted as necessary. (d) By changing the diffusion parameter, the mean lifetime of the agonist-receptor complex, and the size of the interaction field, different amplifications and different speeds of activation can be simulated. (e) Different agonist concentrations can be simulated with different hit rates, $N_{hit}$. (f) Different stoichiometries can be simulated by initial blocking of a certain percentage of receptors and/or effectors.

## Results

**General properties of the model.** The general properties of the model will be described for 3000 receptors and 3000

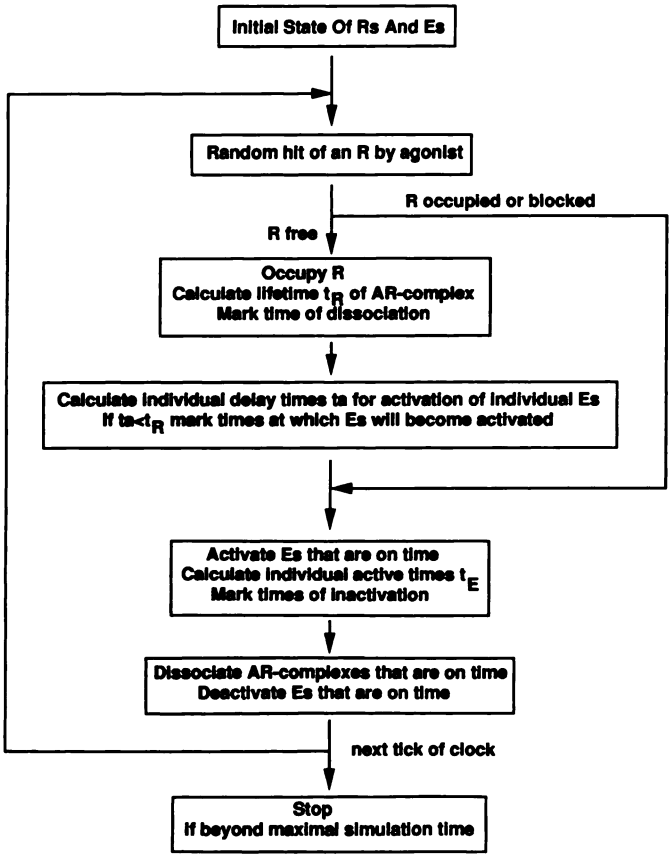


**Fig. 1.** Flow chart of simulation process. See text and Appendix for details. *R*, receptor; *E*, effector; *AR*, agonist-receptor complex.

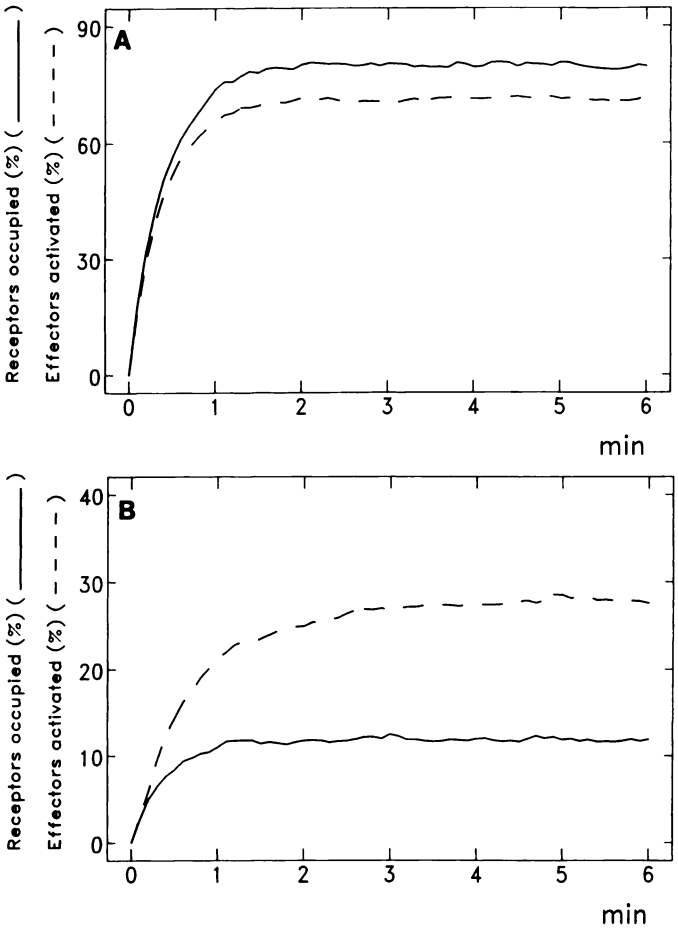


**Fig. 2.** Simulation of receptor occupancy and effector activation as a function of time. The tracings were generated by the simulation program with high (6000 hits/min) (A) and low (200 hits/min) (B) values for $N_{hit}$, corresponding to high and low agonist concentrations. $T_R$ was 2 min, and $T_E$ was 4 min.

effectors, randomly distributed on a sphere, and an interaction field that contains an average of 10 effectors in the vicinity of a receptor. The mean lifetime of agonist-receptor complexes, $T_R$, was set at 2 min, corresponding to the dissociation lifetime of [$^3$H]NECA binding to $A_2$ receptors at 37° (assessed with the methods described in the preceding manuscript, but under the conditions of the adenylate cyclase assay). The mean lifetime of activated effectors, $T_E$, was set at 4 min, corresponding to the lifetime of NECA-stimulated adenylate cyclase after blockade of $A_2$ receptors at 37° (determined as in Fig. 6 of the preceding manuscript). Diffusion parameters were varied; as detailed below, the data on stimulation of adenylate cyclase by $A_2$ adenosine receptors (as obtained in the preceding manuscript) were best reproduced when the diffusion parameter was chosen such that a single agonist-receptor complex activated about three effectors on average.

However, we would like to stress that the parameters of the model can be chosen to simulate any other relationship between the lifetimes of receptor occupation and effector activation and any other diffusion parameter and size of the interaction field.

Fig. 2 shows the time course of receptor occupation and effector activation at low and high hit rates, corresponding to low and high agonist concentrations. Under both circumstances, receptor occupation follows a monoexponential function, with the variations introduced by the random number generators. Effector activation also occurs according to a simple monoexponential curve. The diffusion parameter was set for these simulations such that, on average, each agonist-receptor complex activated three effectors. Similar patterns were obtained at other diffusion parameters (data not shown). A receptor reserve in this model is particularly apparent at low hit rates, with slightly more than 10% occupied receptors resulting in more than 25% activated receptors at equilibrium.

The relationship between receptor occupancy and effector activation at equilibrium for this particular model is shown in Fig. 3. The upward convex shape of the curve documents the presence of a receptor reserve. At low levels of occupancy, there is an average of up to five activated effectors/agonist-occupied receptor. Activation of 50% of the effectors is achieved at 17% receptor occupancy, which corresponds to the transducer ratio of 5.8 (=100/17) found for activation of adenylate cyclase by $A_2$ adenosine receptors in human platelet membranes (see preceding manuscript). Even at maximal receptor occupancy, the percentage of activated effectors does not exceed 80%, in agreement with the observation that maximal activation of adenylate cyclase by NECA reached only 160%, compared with an $E_{max}$ of 200%. This reproduction of the experimental data was dependent on choice of an appropriate diffusion parameter. The same parameter was used for all figures shown in this manuscript.

**Effects of reduction of receptor number.** Photoaffinity labeling of $A_2$ adenosine receptors of human platelet membranes with (*R*)-AHPIA results in an inactivation of up to approximately 85% of the receptors. As described in the pre-

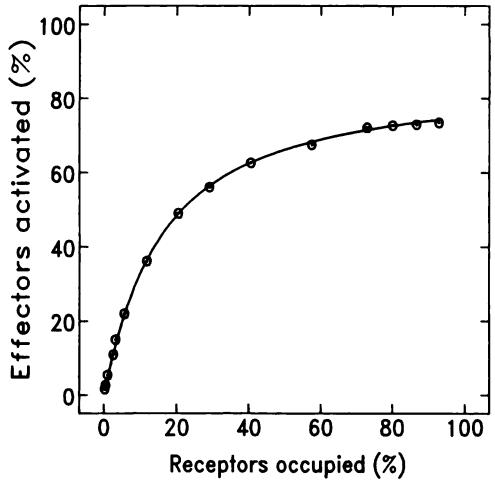


**Fig. 3.** Relationship between receptor occupancy and effector activation. Simulations were done as shown in Fig. 2, with $N_{hit}$ varying from 6 to 20,000 hits/min. Values obtained at "equilibrium" of the simulations were fitted by a hyperbolic equation. Maximal activation of effectors (at 100% receptor occupancy) was 74%, and activation of 50% of the effectors was achieved at 17% receptor occupancy.

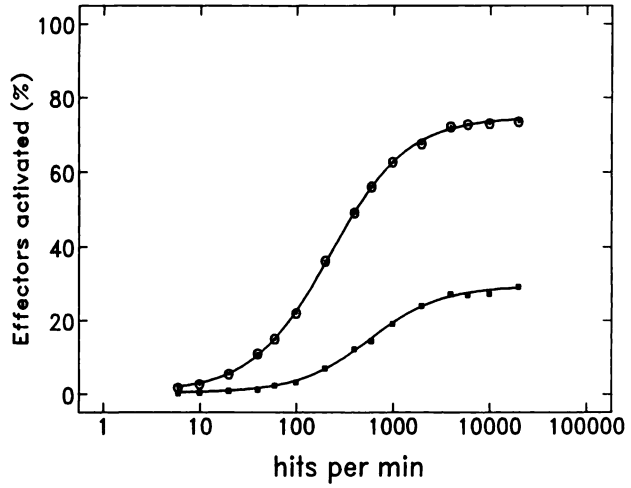


**Fig. 4.** Effects of receptor blockade on effector activation. Effector activation was simulated at different hit rates with 100% (●) or 15% (■) of the receptors available. Simulations were done as shown in Fig. 2. Values obtained at "equilibrium" were fitted to the Hill equation. $EC_{50}$ values and $E_{max}$ values were 200 hits/min and 74% of effectors (100% receptors available) and 590 hits/min and 32% of effectors (15% receptors available), respectively.

ceding manuscript, activation of adenylate cyclase by the residual 15% of receptors differed in the following points from control conditions. (a) The $EC_{50}$ value of NECA was about 3-fold higher and the maximal effect about 2-fold lower, and (b) the kinetics of irreversible adenylate cyclase activation showed an about 2-fold decrease of both the rate and the extent of stimulation.

The effects of reducing the receptor number to 15% were, therefore, tested in the simulation model in order to determine whether this model can account for the experimental observations. The diffusion parameter and the other parameters were set exactly as above.

Fig. 4 shows the concentration-response curve of effector activation by an agonist under conditions of reversible effector activation (i.e., with $T_E = 4$, as above), which simulates the experiment shown in Fig. 4 of the preceding manuscript. Compared with the control curve, activation after blockade of 85% of the receptors is characterized by a slightly more than 2-fold reduction of the maximal effect and an about 3-fold higher $EC_{50}$ value. These results agree with the experimental findings. Next we simulated the experiments shown in Fig. 7 of the preceding manuscript, i.e., the kinetics of quasiirreversible effector activation. The quasiirreversible nature of adenylate cyclase activation in the presence of Gpp(NH)p was simulated by extending the mean lifetime $T_E$ of activated effectors to 100 min. Fig. 5 shows the results of the simulation, which are again in good agreement with the experimental observations. The reduction of the receptor number to 15% resulted in a decrease of both the rate and the extent of effector activation; the half-time of activation is 2.5 times longer, and the extent of activation at equilibrium is reduced about 2-fold. It should be noted that in the corresponding experiment the temperature was 25° (instead of the usual 37°; see preceding manuscript). No attempt was made to account for this alteration of the experimental conditions, which explains the difference in the time scales of activation in Fig. 5, compared with Fig. 7 of the preceding manuscript.

## Discussion

The restricted collision-coupling model presented here accommodates all the experimental findings on stimulation of adenylate cyclase in human platelet membranes by $A_2$ adenosine receptors. It predicts a monoexponential time course of effector activation. The observed transducer ratio of 5.8, as well as the maximal activation of 75% of available effectors, can be simulated by the appropriate choice of parameters. Using these parameters, the experimental findings on the effects of a reduction of the receptor number on both reversible and irreversible activation of adenylate cyclase were correctly predicted.

In addition to simulating the experimental data, the model

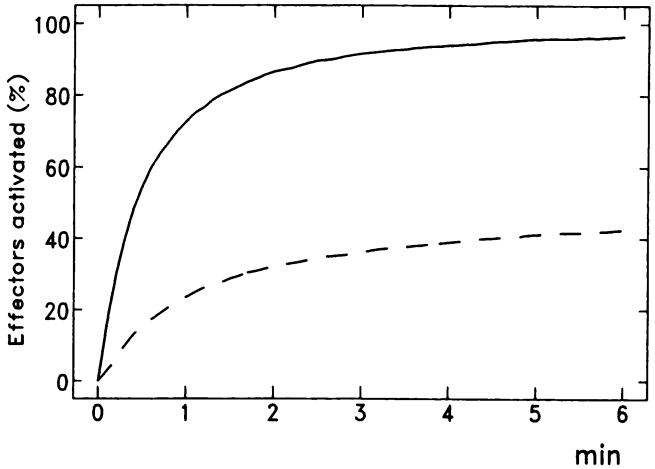


**Fig. 5.** Effects of receptor blockade on kinetics of irreversible effector activation. Simulations were done with 100% of the receptors available (——) or 15% of the receptors available (– – –). Quasiirreversible effector activation was simulated by setting $T_E$ to 100 min. $N_{hit}$ was 6000 hits/min, i.e., corresponding to maximally effective agonist concentrations. Other simulation parameters were as in Fig. 2. Fitting with monoexponential equations gives values for the rate constant $k_{on}$ and the maximal stimulation $E_{max}$ of 1.6 $min^{-1}$ and 97% of the effectors (100% of the receptors available) and 0.65 $min^{-1}$ and 50% of the effectors (15% of the receptors available), respectively.

also accommodates all the predictions of the "agonist model" developed by Black and co-workers (20, 21), which was used to interpret the data in the preceding manuscript. The transducer ratio can be read directly from the graph in Fig. 3 as the ratio of total receptors (100%) to receptors leading to activation of 50% of the effectors. With this transducer ratio, our model generates the same kind of concentration-response curves as a function of available receptors (compare Fig. 4 of this manuscript with Fig. 4 of the preceding one). Thus, our discrete simulation model is in agreement with the model of Black and co-workers (20, 21). However, the latter describes only equilibrium data, whereas our model can also be investigated in kinetic terms.

Two models that describe the kinetics of receptor-effector coupling have been validated by Levitzki and co-workers (6, 8) from experiments investigating the effect of a reduction of the receptor number on the irreversible activation of adenylate cyclase. These are the noncatalytic precoupled and the catalytic collision-coupling models (6, 8). However, the behavior of both seemingly contradictory models can be simulated by the restricted collision-coupling model proposed here and, thus, the kinetic predictions of these models represent only two extremes of our model. In the absence of a receptor reserve, as in the extreme case when a receptor can activate no more than the nearest effector, a reduction of the receptor number results in a proportional reduction of the extent of effector activation, with no effect on the rate, i.e., the model follows the kinetic predictions of the precoupled model. This can be simulated by using either a slow diffusion parameter or a short lifetime of the agonist-receptor complexes. On the other hand, with a large receptor reserve, simulated by either a long lifetime of the agonist-receptor complexes or a high diffusion parameter, a reduction of the receptor number decreases the rate but has little effect on the extent of effector activation, i.e., the model behaves according to the collision-coupling model.

In fact, the $\beta$-adrenergic receptor of turkey erythrocyte membranes, which is proposed to activate adenylate cyclase according to the collision-coupling model (6), activates adenylate cyclase to a much larger extent than the $A_2$ adenosine receptor (7), which is proposed to conform to the precoupled model (8). This observation would be consistent with a receptor reserve for the $\beta$-adrenergic receptor but not the adenosine receptor in these membranes. Consequently, in both cases the observed behavior after reduction of the number of respective receptors would be predicted by the restricted collision-coupling model.

Likewise, a receptor reserve can be deduced from the data on adenylate cyclase stimulation by glucagon receptors in liver membranes, a system also proposed to conform to the collision-coupling model (22).

The key features of our model are (a) catalytic activation of effectors by receptors (6, 11) and (b) restrictions of the interaction between receptors and effectors. These restrictions are due to slow diffusion of receptors (12) and effectors, limiting effector activation to a small area around an agonist-occupied receptor. Using these features, it appears possible to adequately simulate the coupling between different receptors and adenylate cyclase.

The model also allows a determination of the amplification, which occurs at the step between receptor occupation and effector activation. This amplification can be read from the initial portion of the receptor occupancy versus effector activation plot (Fig. 3); one $A_2$ adenosine receptor can lead to activation of up to five effectors. The amplification is thus small, compared with the value of several hundred reported for the rhodopsin-phosphodiesterase system (23), but is in the same range as the values reported for $\beta$-adrenergic receptor-adenylate cyclase coupling in reconstituted systems (4).

In summary, the restricted collision-coupling model is a discrete mechanistic model characterized by the following principles. Agonist-occupied receptors activate effectors sequentially by colliding with them in a slow diffusion process. The number of effectors that become activated by an agonist-occupied receptor and, hence, the presence of a receptor reserve is determined by the mean lifetime of the agonist-receptor complex and by the mobility and density of receptors and effectors. The data obtained with these simulations are compatible with kinetic and equilibrium experiments on $A_2$ adenosine receptor-adenylate cyclase coupling described in the preceding manuscript. In addition, the model also accommodates the general descriptions of various other models. It provides a mechanistic basis and a mathematical description for the simulation of receptor-effector coupling.

## Appendix

In the mathematical model, the membrane is represented by the unit sphere

$$S = \{(x,y,z): x^2 + y^2 + z^2 = 1\} \quad (1)$$

The center of this sphere is $M$ (see Fig. 6).

Any point $P$ on this sphere is given by cartesian coordinates $P = (x,y,z)$ or spherical coordinates $P = (\varphi,\theta)$, which are related to each other by

$$(x,y,z) = (\cos\varphi \cdot \sin\theta, \sin\varphi \cdot \sin\theta, \cos\theta)$$

$$\varphi = \arctan(y/x), \ \theta$$

$$= \arccos(z), \ 0 \le \varphi < 2\pi, \ 0 \le \theta < \pi \quad (2)$$

The surface distance between two points, $P_1 = (x_1,y_1,z_1)$ and $P_2 = (x_2,y_2,z_2)$, on the sphere $S$ is defined as the length of the geodesic arc connecting these points (see Fig. 6); i.e., the shorter segment of the great circle passing through $P_1$ and $P_2$. Because

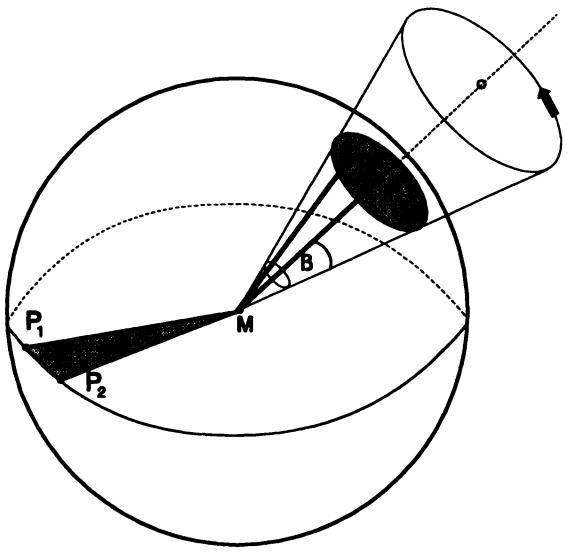


**Fig. 6.** Representation of the mathematical model.

$S$ is the unit sphere, this equals the angle $\alpha$ between the radii $MP_1$ and $MP_2$ and is calculated as

$$\alpha = \arccos(x_1x_2 + y_1y_2 + z_1z_2) \text{ or} \quad (3)$$

$$\cos\alpha = x_1x_2 + y_1y_2 + z_1z_2$$

Any receptor is a point $R = (x_R,y_R,z_R)$ on the sphere $S$. Rotating a vector at $M$, which has an angle of $\beta$ with the radius $MR$, generates a cone with aperture angle $2\beta$ (see Fig. 6). This cone defines a circular area $A$ on the sphere (the spherical surface of a spherical segment) with center $R$. Any effector $E = (x_E,y_E,z_E)$ on the sphere lies inside this area if the angle between the radii $MR$ and $ME$, i.e., the surface distance between $R$ and $E$, is less than $\beta$. Using Eq. 3, this is equivalent to

$$x_Rx_E + y_Ry_E + z_Rz_E > \cos\beta \quad (4)$$

because the function $\cos(x)$ is decreasing for $0 \leq x \leq \pi$.

The size of area $A$ can be calculated as

$$A = 2\pi\cdot(1 - \cos\beta). \quad (5)$$

If $N$ effectors are uniformly distributed on the sphere $S$, their density is

$$\delta = N/4\pi \quad (6)$$

because $4\pi$ is the area of the whole unit sphere. Hence, the number of effectors found in a circular area $A$ on the sphere, as described above, is governed by the binomial distribution $B$ with parameters $N$ and

$$p = \delta\cdot A = (1 - \cos\beta)/2 \quad (7)$$

i.e., the probability $w$ of finding $k$ effectors inside $A$ is

$$w = n!/(k!(n - k)!)\cdot p^k(1 - p)^{n-k}$$

Expectation value $E[B]$ and variance $VAR(B)$ are

$$E[B] = N/2\cdot(1 - \cos\beta) \quad (8)$$

$$VAR(B) = N/4\cdot(1 - \cos^2\beta)$$

Eqs. 8 are used to determine some simulation parameters.

Due to restrictions in size and speed of the computers available to us, it was not possible to simulate the Brownian motion of the agonist-receptor complex directly. We used a different approach. Depending on its lifetime and diffusion rate, any agonist-receptor complex can reach a certain number of effectors on the membrane. Because Brownian motion is isotropic, we simulated this by defining a circular area, of the type described above, for each receptor as the maximum region it may cover during its lifetime as an agonist-receptor complex. In our simulations, the agonist-receptor complex can only interact with effectors inside this area; hence, we call this region the interaction field of the receptor. This area should be large enough to allow simulations with varying lifetimes and diffusion rates. We used $N = 3000$ effectors on the sphere and $m = 10$ effectors as the average number in the interaction field of an agonist-receptor complex. Using Eqs. 8,

$$m = N/2\cdot(1 - \cos\beta) \text{ or } \cos\beta = 1 - (2m)/N$$

this determines the size of the circular area around each receptor, such that one finds $m = 10$ effectors inside the area, on the average.

At the beginning of the simulation, the interaction field is scanned for each receptor to build a list of all effectors inside. This list contains the numbers of the effectors and their surface distance to the receptor. Because effectors are randomly distributed on the sphere, the numbers of effectors in these lists vary. For technical reasons, we restricted the size of these list to $m + 1$ SD, as can also be calculated from Eqs. 8. Hence, for $N = 3000$, $m = 10$, the size of the list was restricted to 14.

After occupation of a given receptor by agonist, the list of the interaction field of the receptor is utilized to determine those effectors inside the interaction field, which will be activated by this agonist-receptor complex, and, additionally, when this happens. According to our assumption, the agonist-receptor complex moves around and, eventually, gets near enough to an effector inside the interaction field to activate this effector. The time interval, $t_a$, between occupation of receptor by agonist (i.e., start of the lifetime of the agonist-receptor complex) and activation of effector is a random variate depending on the surface distance, $a$, between receptor and effector and the diffusion parameter, $D$, of the agonist-receptor complex. As approximation for the random distribution of $t_a$, we chose the first-passage time distribution of one-dimensional brownian motion (10).

$$f(t) = \frac{a}{\sqrt{2\pi D}}\, t^{-3/2}e^{-(a^2/(2Dt))} \quad (9)$$

This is the distribution of the first time of reaching distance $a > 0$ for a Brownian particle starting at the origin and moving back and forth on a straight line.

Using a random generator for this distribution and the information from the list of the interaction field, the program generates a time interval, $t_a$, for all effectors in the interaction field of the receptor at the moment the receptor becomes occupied. The time $t_a$ is compared with the individual lifetime $t_R$ of the agonist-receptor complex. The complex will only activate an effector if $t_a$ is less than $t_R$, i.e., if the agonist-receptor complex reaches an effector at all during its lifetime, and if no other agonist-receptor complex will do this earlier.

Although the mean lifetime of agonist-receptor complexes, $T_R$, was set at 2 min, the diffusion parameter, $D$, was adjusted by trial and error such that one agonist-receptor complex would activate approximately three effectors, on the average. Hence, the average area of interaction is much smaller than the interaction field, as constructed above. In this way, potential boundary effects are avoided during the simulations. Of course, if $T_R$ and/or $D$ are increased too much, one has to enlarge the size of the interaction field as well. Nevertheless, this size is not a restricting factor of our model and can be adjusted to individual needs.

The simulation program is available upon request. The following versions are available: for VAX minicomputers under VMS, for IBM mainframes under VM, and for IBM PCs written in Turbo-Pascal. Because no special features of Turbo-Pascal are used, the program is easily adapted to different machines. In spite of the memory restrictions on personal computers, the Turbo-Pascal version works with up to 2000 receptors and 2000 effectors. The VAX minicomputer and IBM mainframe versions can process much larger simulations.

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