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# QUANTITATIVE ASPECTS OF A UNIFIED MODEL OF DIFFUSION MEDIATED RECEPTOR–CYCLASE COUPLING

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A quantitative model is presented of diffusion mediated coupling of adenylate cyclase to multivalent plasma membrane receptors which accounts for a wide range of phenomena including non linear occupation–activation plots with either positive or negative second derivatives, spare receptors, silent receptors, and negative and positive binding cooperativity. A non linear least square fit of the predicted equation for cyclase activation to available data predicts translational diffusion coefficients in the range of $(10^{-10}-10^{-11})$ cm$^2$/s.

Considerable quantitative data is now available relating various biochemical correlates of cellular activity to the physical chemistry of cell surface events. Of particular interest have been studies of adenylate cyclase activity as a function of the concentration of various ligands, including peptide hormones, neurotransmitters and antigens, which are specifically bound to receptors on the plasma membrane. The results, which bear on mechanisms operative in the early stages of transmembrane signal transmission, often reveal a highly non linear relation between binding and activation, suggestive of a transductive process which may act either to amplify small environmental fluctuations, or to stabilize the cell against them. Thus in many instances the activity of the enzyme, as measured by c AMP accumulation, leads binding and may be very nearly at its peak with only a few percent of the receptors occupied ("spare receptors") [1] while in other instances activity lags binding [2]. In addition to these steady state observations, kinetic results indicate that in some systems binding continues after activation has attained the steady state [3,4] suggesting that some binding sites are acceptors (or "silent" receptors) rather than true receptors.

An interpretation of data of this sort in terms of specific models is made difficult by lack of adequate structural knowledge. Moreover, cyclase activity is integrated with a variety of cellular events, such as changes in the surface modulating cytoskeletal apparatus, in guanyl cyclase and phosphodiesterase activities and so forth, all of which are likely involved in large scale positive and negative feedback loops affecting both receptors and cyclase. Nevertheless the data impose minimal requirements which a model must meet: qualitatively in terms of having to conform to a range of experimentally observed functional relations, and quantitatively in terms of the parameter values required to fit these relations. During the past few years a number of investigators have shown how the analysis of binding data can place limits on the number of possible models of molecular organization [5,6] and that the floating receptor model in particular can account for many qualitative features of the observed activation-occupation plots [7]. In this paper we pursue the implications of a diffusion amplified mechanism of the coupling of cyclase to *bivalent* regulatory receptors. We show that the model can be developed in terms of two lumped parameters, and that estimates of the parameters obtained by non linear least square fits of a variety of data lead to reasonable values for receptor diffusion coefficients in the plane of the membrane.

First consider the non linear relation between binding and activation. A high degree of activation can be achieved at a relatively low degree of binding if

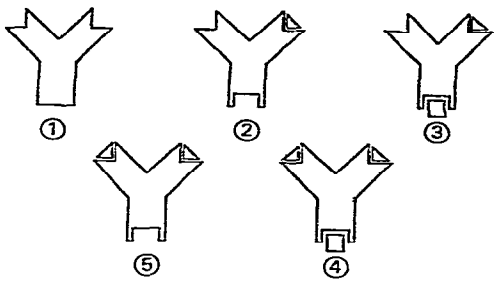


Fig. 1. States of a receptor which is bivalent for hormone and monovalent for cyclase. The equations in the text include only states (1)–(4) and therefore represent a special case of the model; viz the one in which the second receptor site for hormone is induced by cyclase binding. ($k_1$, $k_{-1}$) are the forward and reverse rate constants of hormone interacting with an unoccupied receptor, ($k'_1$, $k'_{-1}$) are the rate constants for hormone interacting with a singly bound receptor and ($k_2$, $k_{-2}$) are the rate constants for cyclase interacting with an active receptor. Dissociation of hormone from a cyclase bound receptor occurs with rate constant $k_3$ and is assumed to lead to immediate dissociation of the enzyme

a bimolecular chemical reaction between cyclase and receptor is required to activate the cyclase (see below). In fact cell fusion experiments indicate that for $\beta$ adrenergic receptors at least, there is no permanent coupling between receptor and cyclase [8], and consequently it seems natural to suppose that cyclase is activated either by direct interaction with hormone bound receptor or indirectly through molecular messengers [9]. In either case activation depends on a bimolecular reaction and is therefore quadratically non linear in some *function* of the hormone concentration. From this point of view, results which suggest spare receptors are an expected consequence of membrane fluidity *when the available number of cyclase molecules is not in large excess over the number of receptors.* It is evident that an important aspect of such a hypothesis is that activation is sensitive to membrane viscosity, and consequently the magnitude of the diffusion coefficient is one of the determinants of amplification.

Although diffusion mediated non linear receptor cyclase coupling as outlined above is sufficient to describe steady state occupation-activation plots in which activity leads binding, it incorporates neither silent receptors, nor results in which activation lags binding. A simple additional hypothesis is that the receptor has access to several states, each associated with a different ability to activate cyclase. For example the receptors might be multivalent and thus have access to several states of binding, or equivalently aggregates of several monofunctional receptors might exist in a clustered state [10,11]. Alternatively hormone may induce clustering, just as cell surface antigens and other molecules are clustered by appropriate ligands [12].

For the former case, with bivalent receptors (or aggregates of two monovalent receptors which are in existence prior to exposure to hormone) which bind hormone cooperatively, an analytic expression for activation as a function of occupation is readily obtained in the steady state. Specifically, for the model in fig. 1, $x$, the fraction of receptors in state 3, is the solution to (see Appendix)

$$x^2\alpha_1(1 + K'H/2)[\alpha_3 + KH(1 + K'H/2] - x\{1 + \alpha_1 KH(1 + K'H/2) + \alpha_1\alpha_2\alpha_3 + KH[1 + \alpha_1\alpha_2(1 + K'H/2)]\} + \alpha_1\alpha_2 KH = 0, \quad (1)$$

where $\alpha_1 \equiv k_2 C_0/(k_{-3} + k_{-2})$; $\alpha_2 \equiv A_0/C_0$; $\alpha_3 \equiv k_{-3}/k_{-1}$; $K \equiv k_1/k_{-1}$ and $K' \equiv k'_1/k'_{-1}$. $C_0$ and $A_0$ are the total number of receptors and cyclase molecules respectively, and $H$ the free hormone concentration in the vicinity of the cell surface. The latter is generally in large excess over the concentration of receptors and is therefore approximated by the total hormone concentration. Actually $A_0$ might more accurately be interpreted as the number of cyclase molecules available for interaction with receptors. For example a possibility consistent with the model of Rendell et al. [13] would be to interpret $A_0$ as the fraction of the total cyclase pool which is bound by $G_{pp}(NH)_p$.

If $r$ denotes the activity of state four relative to that of state three, the total cyclase activity, $A_c$, is given by

$$A_c = C_0 x(1 + rK'H/2). \quad (2)$$

The concentration of bound hormone, $B \equiv C_2 + C_3 + 2C_4$ is also expressible in terms of $x$ (Appendix)

$$B = C_0 x\{1 + K'H + [\alpha_1(\alpha_2 - x - xK'H/2)]^{-1}\}. \quad (3)$$

A plot of the fraction maximum activation against

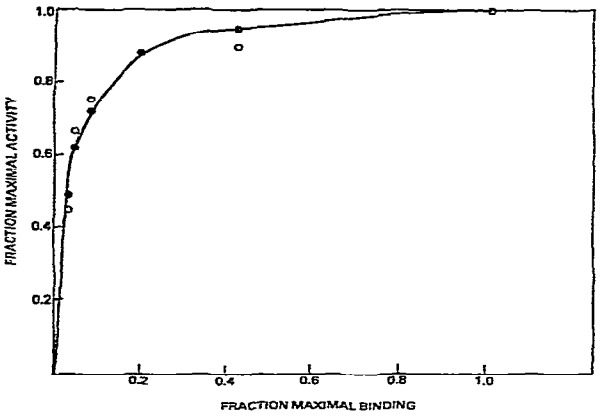


Fig. 2. Cyclase activation by Lys–vasopressin binding to plasma membrane receptors on porcine renal medullaty cells (–•–) experimental [11]; (–o–), calculated.

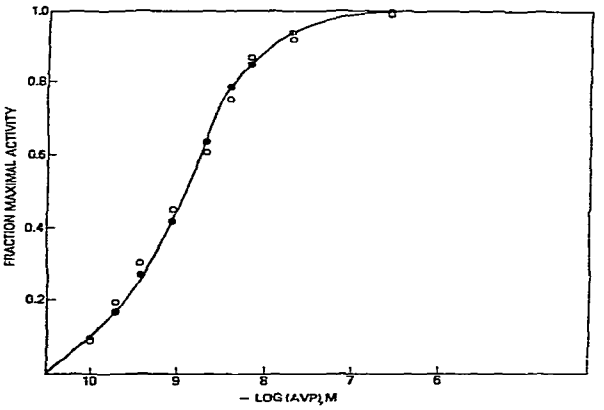


Fig. 3. Activation by Arg vasopressin binding to receptors on bovine renal medullary cells (–•–) experimental [12]; (–o), calculated.

fraction maximum binding can be obtained by dividing eqs. (2) and (3) by their maximum values, $A_c^*$ and $B^*$, where for $K' \neq 0$,

$$A_c^* \equiv \lim_{H\to\infty} A_c = \begin{cases} rC_0 & A_0 > C_0 \\ rA_0 & A_0 < C_0 \end{cases} \tag{4a}$$

and

$$B^* \equiv \lim_{H\to\infty} B = \begin{cases} 2C_0 & A_0 > C_0 \\ C_0 + A_0 & A_0 < C_0 \end{cases} \tag{4b}$$

If c AMP accumulation is linearly proportional to the amount of active cyclase (which will be true if Michaelis-Menton kinetics are satisfied) then the quantity usually measured; viz, the fraction maximum cyclic AMP accumulation, will be equal to the fraction maximum cyclase activity, $f_A (\simeq A_c/A_c^*)$.

For the special case in which $K' = 0$, the ratio of eq. (3) to eq. (2) is

$$\frac{B}{A_c} = \frac{1 + \alpha_1(\alpha_2 - A_c)}{\alpha_1(\alpha_2 - A_c)}. \tag{5}$$

In this case $A_c^*$ is the solution to eq. (5) with $B = C_0$. Although eq. (5) involves five parameters, ($k_{-1}$, $k_{-2}$, $k_2$, $A_c$, $C_0$) the analysis indicates that only two combinations of these determine the shape of the curve; viz, the ratio of cyclase molecules to receptor molecules ($\alpha_2$) and the ratio of the rate at which complexes form to the rate at which they dissociate ($\alpha_1$). Numerical simulations indicate that a plot of $f_A$ against $f_B$ ($\cong B/B^*$) based on eq. (5) cannot cross the line $f_A = f_B$, but remains above it, approaching it for values of $\alpha_1$ and $\alpha_2$ sufficiently greater than 1, so that this simple equation cannot describe results in which binding leads activation.

As an example of the coupling rates and diffusion coefficients which are predicted by a non linear least square fit of the model to activation data, I have applied eq. (5) to activation of procine [14] and bovine [15] renal medullary cyclase by vasopressin. This allows determination of $\alpha_1$, and if $k_{-1} > k_{-2}$, the coupling rate ($k_2 C_0$) required by the model can be estimated. For these determinations, the least square estimate of $\alpha_1$ was insensitive to the presence or absence of $K'$. For the porcine system, $k_{-1} = 7.5 \times 10^{-4}$ $s^{-1}$ [14] and $\alpha_1 = 30$ (fig. 2) implying a coupling time $(k_2 C_0)^{-1} < 50$ s. For the bovine system, using $k_{-1} = 7 \times 10^{-3}$ [16] $s^{-1}$, the least square estimate of $\alpha_1$ was 80, (fig. 3) implying $(k_2 C_0)^{-1} < 2$ s. These are upper bounds since $k_{-2}$ has been neglected. Interestingly, Bergman and Hechter [17], using an entirely different model, have obtained a thirty second coupling delay.

The extent to which the order of magnitude difference between the procine and bovine system is real, rather than the result of different assays for determining

$k_{-1}$ (the former result is obtained by a direct binding assay, whereas the latter relies on cyclase activation kinetics) is not known. If real, the difference in the estimated coupling delays implies a substantial difference in membrane fluidity, and therefore translational diffusion coefficients. With regard to the latter, using the least square estimates of $\alpha_1$, the sum of the translational diffusion coefficients of receptor plus cyclase can be estimated by [18,19]

$$D = \alpha_1(k_{-1} + k_{-2})d^2 \frac{\ln(d/r_0)}{2\pi},$$

where $d$ is the mean spacing between the centers of mass of receptor and cyclase when they are in random motion, and $r_0$ is the distance between their centers of mass in the complexed form. With $2 \times 10^4$ receptors per cell and a comparable number of cyclase molecules, a cell radius of 10 $\mu$m and $r_0 = 10$ Å, I obtain $D = 2 \times 10^{-11}$ cm$^2$/s for the porcine system and $4 \times 10^{-10}$ cm$^2$/s for the bovine system. Although these diffusion coefficients, estimated by fitting the model, are within the range of directly measured values [20,21], they should be interpreted as lower bounds because of the omissions of $k_{-2}$ and the orientational requirements for reaction. In three dimensions the latter may be as low as $10^{-2}$, but on a membrane, with two orientational degrees of freedom constrained, it is expected to be considerably larger [18].

For vasopressin binding to porcine renal medullary cells, activation is a more sensitive function of bound ligand than in the bovine system and this implies a larger value of $\alpha_1$ and perhaps a smaller value of $\alpha_2$ (the latter could not be determined significantly). Thus a decrease in fluidity will be reflected in an increased value of $k_2$, leading to an apparently larger number of spare receptors. A decrease in $k_{-2}$ and/or $k_{-1}$ would have the same effect. $\alpha_1$ was also determined for several other systems including glucagon binding to rat liver cells [22] and thyroid stimulating hormone binding to thyroid cells [2]; the estimated values all being within an order of magnitude and ranging from a low of 5 in the case of TSH to a high of 80 for vasopressin. It should be noticed that because coupling depends on $k_{-1}$ (through $\alpha_1$), the model predicts that hormone analogues with lower affinities for receptors will be less effective in activating the cell than hormone, even when the same number of receptors are bound.

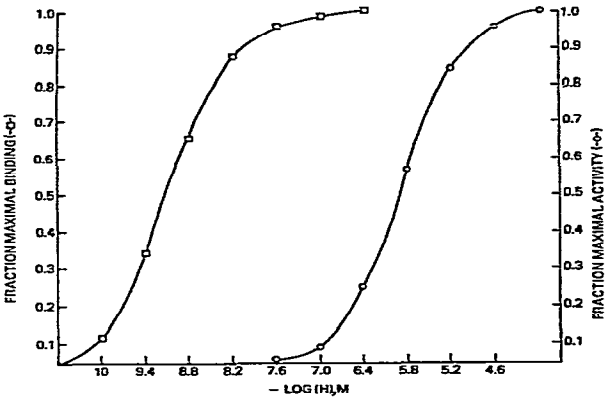


Fig. 4. Simulation of the full model with binding leading activation. $\alpha_1 = 0.09$, $\alpha_2 = 0.06$, $\alpha_3 = 0.38$, $K = 10^9$ M$^{-1}$, $K' = 6 \times 10^7$ M$^{-1}$, $r = 95$.

In the more general case in which $K' \neq 0$, binding may lag or lead activation, depending on the relation between $K'$ and $K$, and the value of $r$. An example in which activation remains low until binding is very nearly complete and the required parameter values is shown in fig. 4. The relation follows as the result of a doubly bound receptor being associated with a state of higher cyclase activity than one which is singly bound. Alternatively, if all or most of the activity were in the first step, activation kinetics would be very nearly complete with binding still far from completion and the binding sites would be considered acceptors. In such a circumstance, the second binding event, though not contributing directly to activity, would still play an important role since two dissociations would be required before the receptor could deactivate.

The inclusion of desensitization within the mobile receptor concept has been indicated previously for a somewhat different model [23], but similar ideas apply here. Among the possibilities, if state 3 were active and state four inactive but with a lower binding affinity for hormone, then relatively low concentrations would favor state 3, whereas higher prolonged concentrations would favor state 4. Alternatively, state four could be active and still lead to desensitization if it favored large aggregate formation followed by endocytosis or shedding; a possibility which can be,

but has not been included explicitly in the model.

More complicated coupling models are of course also possible, including models of hormone induced clustering in which aggregates of various sizes activate cyclase to different degrees. When both hormone and receptor are multivalent, large scale redistribution of receptors can occur as the result of physical bridges being formed by the hormone between receptors. However in this case the concentration of cross links is expected to rise, and then fall again at high concentrations when all sites become bound by hormone [24]; The apparent monotonic increase in activation in many hormone systems suggests that if clustering occurs and is related to activation, it is not induced as the result of the hormone bridging receptors, although other clustering mechanisms are of course possible. In any case binding data over a much wider range would be desirable in order to reach firmer conclusions about this distinction.

More generally some insight into the factors controlling the non linearity of occupation activation plots can be obtained by temperature dependent studies. A temperature increase will increase the diffusion coefficient (and hence, $k_2$) both because of a decrease in viscosity and an increase in thermal energy. Thus theory predicts that decreasing temperature would lead to apparently fewer "spare receptors". In a more biological context, the cell can amplify the binding signal (i.e. appear to have more or less spare receptors) not merely by changes in receptor number (which affects both $\alpha_1$ and $\alpha_2$) but by any change in membrane composition which leads to a viscosity change.

In summary, the model presented here indicates that a wide range of phenomena including non linear occupation activation plots with either positive or negative second derivatives, spare receptors, negative and positive binding cooperativity, and the decreased activity of analogues can be unified with a simple model whose only requirement is the existence of receptors which are affectively multivalent for ligand and normally uncoupled from cyclase. It is also flexible enough to qualitatively incorporate desensitization and the silent receptor phenomenon. Least square fits of the model to available data predict reasonable values of coupling rates and translational diffusion coefficients. Additional observations which floating receptor models do and do not explain have been reviewed elsewhere [25].

## Appendix

*Steady state solution to the kinetic model* (fig. 1)

The equations in the text include only states 1—4 and therefore represent a special case of the model. Solution to the more general model is of course possible and introduces greater flexibility. However, the additional adjustable parameters which would be introduced seem to be unnecessary to explain the phenomena considered in this paper. Denoting the concentration of the $i$th state ($i = 1, 2, 3, 4$) by $c_i$, the differential eqs. governing their interconversion are:

$$dc_1/dt = -k_1Hc_1 + k_{-1}c_2 + k_{-3}c_3, \tag{A.1}$$

$$dc_2/dt = k_1Hc_1 - (k_{-1} + k_2A)c_2 + k_{-2}c_3, \tag{A.2}$$

$$dc_3/dt = k_2Ac_2 - c_3(k_{-3} + k_{-2} + k'_1H) + 2k'_{-1}c_4, \tag{A.3}$$

$$dc_4/dt = k'_1Hc_3 - 2k'_{-1}c_4. \tag{A.4}$$

$A$ is the free enzyme concentration and $H$ the free hormone concentration (the latter is assumed to be in large excess over the concentration of receptor sites, and therefore well approximated by the total concentration). In addition to eqs. (1)–(4), if the *total* receptor concentration, $c_0$, is constant, then

$$c_0 = c_1 + c_2 + c_3 + c_4 \tag{A.5}$$

and only three of the four differential equations are independent. The equations are non linear because $A$ is a function of the $c_i$ and they must in general be solved numerically. However, closed form solutions under steady state conditions are readily obtained. Setting the left hand sides equal to zero, solving (A.2)—(A.4) in terms of $c_1$ and using eq. (A.5) one finds that

$$c_1 = (c_0 + \alpha_1\alpha_3A)/Z, \tag{A.6}$$

$$c_2 = KHc_0/Z, \tag{A.7}$$

$$c_3 = KH\alpha_1A/Z, \tag{A.8}$$

$$c_4 = KK'H^2\alpha_1A/2Z = (K'H/2)c_3, \tag{A.9}$$

where

$$Z = 1 + A\alpha_3\alpha_1/c_0 + KH(1 + A\alpha_1/c_0 + K'HA\alpha_1/2c_0), \tag{A.10}$$

$$\alpha_1 \equiv k_2 c_0/(k_{-2} + k_{-3}), \quad \alpha_3 \equiv k_{-3}/k_{-1},$$

$$K \equiv k_1/k_{-1}, \quad K' \equiv k_1'/k_{-1}'.$$

Eqs. (A.6)–(A.9) can be written in terms of $A_0$ rather than $A$ by noting that

$$A_0 = A + c_3 + c_4. \tag{A.11}$$

The equation for $c_3$ then becomes

$$c_3 = \alpha_1 A_0 KH/[Z + \alpha_1 KH(1 + K'H/2)], \tag{A.12}$$

where

$$Z = 1 + \alpha_1\alpha_3[\alpha_2 - x(1 + K'H/2)] + KH\{1 + \alpha_1[\alpha_2 - x(1 + K'H/2)][1 + K'H/2]\},$$

$\alpha_2 \equiv A_0/c_0$ and $x \equiv c_3/c_0$.

Eq. (1) in the text follows upon rearranging eq. (A.12).

According to fig. 1, the bound hormone concentration, $B$, is given by

$$B = c_2 + c_3 + 2c_4. \tag{A.13}$$

Eq. (3) in the text follows from eq. (A.13) by utilizing eqs. (A.7)–(A.9) and (A.11).

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