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HORMONE RECEPTOR MOBILITY AND CATECHOLAMINE BINDING IN MEMBRANES. A Theoretical Model

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[^{3}H]-Catecholamine binding to intact cells, isolated cell membranes, and to several isolated macromolecules has been shown by several laboratories to be neither stereospecific nor inhibited by known β-antagonists. Since additional evidence indicates that this binding is not an artifact (i.e. due neither to the binding of a catecholamine oxidation product nor hormone binding to a catabolic enzyme such as COMT), the question remains as to whether this represents binding to a bona fide membrane receptor. Because all ligands which bind strongly or compete for this binding possess a catechol group, one possible explanation is that the binding affinity is primarily determined by the catechol moiety, whereas the correct stereoisomer of the side chain is necessary to activate the receptor. Thus, although binding is a necessary condition for hormone action, the necessary and sufficient condition for activation of adenyl cyclase is both the catechol group and the correct stereoisomer of the side chain.

A theoretical model is developed here to provide a quantitative basis for this hypothesis. This model extends the current concept of distinct subunits in the adenyl cyclase system by separating the receptors from the catalytic sites and placing them at separate locations within the membrane. Utilizing the spare receptor model of Furchgott, and the mobility of macromolecules within a "lipid sea," the appropriate equations to predict both hormone binding and enzyme activation are derived. Using the observed affinity constants from catecholamine binding studies, it is then shown that this model can predict the experimental observations and hence explain the apparent dichotomy arising from binding and enzyme activation studies.

INTRODUCTION

In recent years evidence from both theoretical and experimental biology has implicated the cell membrane in playing an integral part in the mechanisms of regulation of cellular activity (1, 2). Perhaps two of the most instrumental developments in fostering this hypothesis have been: First, the discovery of the hormone-sensitive adenyl

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Abbreviations: ATP, adenosine triphosphate; cyclic AMP, cyclic adenosine 3′, 5′-monophosphate; GTP, guanosine triphosphate; COMT, catechol-o-methyl transferase; R, hormone receptor, C, enzyme catalytic component; L, ligand; A, agonist; I, antagonist; N, binding inhibitor; brackets, [ ], implies concentration.

cyclase system by Sutherland and Rall (3), thereby associating this specific biochemical event with membrane function; and second, Singer and Nicholson's fluid mosaic model of the plasma membrane (4) which gave emphasis to the dynamic properties of membrane structure. Subsequent research by many laboratories on adenyl cyclase demonstrated a very complex system, particularly with respect to the primary events leading to the enzymatic activity (5, 6). Thus, while the biochemical function of this system was quickly established (i.e. the catalysis of the conversion of ATP to cyclic-AMP), as was the realization that the enzymatic activity was regulated by circulating hormones, the specificity of this regulation was, and is still poorly understood.

Hormone receptors were quickly postulated to be responsible for the tissue specificity, i.e. although many different hormones are capable of stimulating adenyl cyclase, only a fixed few of these effector molecules are active on any given tissue. These hormone receptors are considered to be complex protein components of the cell membrane, accessible from the exterior side of the cell, which bind specifically with the hormone and initiate those events leading to the cellular response. Such concepts led Robison et al. (6) to propose the first membrane model of the adenyl cyclase system. This model emphasized a two component system, i.e. a receptor-regulatory part exposed to the outer surface and a catalytic unit on the inner side of the membrane. Separation of adenyl cyclase into separate components on which effector molecules can act was very significant to further understanding this enzyme system. However, as radioactive hormones of high specific activity have become available, direct studies of the hormone-receptor interaction have raised many questions which indicate a more complex system.

Binding studies that focus on the primary events in the adenyl cyclase system have shown that most hormone binding does not correlate exactly with stimulation of the enzymatic activity. Although, for a number of reasons this inconsistency is most often associated with the catecholamines, it is a fact that all hormones (that activate the adenyl cyclase system) that have been studied do not show a simple and direct relationship between hormone binding and cyclase activation. For example, Rodbell and associates (7–11) have shown that the binding of glucagon to rat liver plasma membranes has an apparent affinity similar to the activation of adenyl cyclase, is reversible, and is inhibited by certain compounds which also inhibit the enzyme activity. However, the kinetics for binding are not in agreement with the almost instantaneous activation of adenyl cyclase. Furthermore, under the identical conditions of stimulation, or specifically, in the presence of ATP, there is a 50% decrease in binding (7–9), suggesting that the concentration dependence of binding does not agree with enzyme activation. Similarly, in the presence of GTP it has been shown that while the binding affinity for glucagon is reduced, the cyclase activity is observed to increase without any apparent changes in the observed concentration dependence. Moore and Wolff (12) have shown that the binding of [^{3}H] thyroid-stimulating hormone (TSH) is rapid and reversible and appears superficially to correlate well with enzyme activity. However, as with glucagon, nucleoside triphosphates inhibit binding while at the same time stimulating adenyl cyclase activity. In addition, they observed that phospholipase A and filipin inhibit cyclase activity yet enhance binding. After considering a number of different conditions, they were forced to conclude that there is not a direct correlation between hormone binding and activation, and that any relationship is complex. Similar discrepancies have been noted by Lang and Schwyzer for ACTH binding and activation (13, 14).

As with the peptide hormones, the catecholamines have received considerable characterization in terms of binding and activation of adenyl cyclase (15–20). Because

these molecules are of low molecular weight compared with the peptide hormones, more detailed information using stereoisomers and agonists or antagonists is known in terms of both binding and activation. This field has recently been reviewed (21, 22), however, rather than acknowledging that the discrepancies between catecholamine binding and cyclase activation may in some way be related to the discrepancies observed for the peptide hormones, it was concluded that the catecholamine binding is nonspecific. To be sure, the fact that catecholamine binding is not stereospecific nor inhibited by known β-antagnoists is surprising, yet this may be no more serious than the discrepancies observed for the peptide hormones. We have shown in this laboratory, as have others, that this binding in the erythrocyte membrane is not due to the artifacts as proposed. Thus, catecholamine binding to these membranes can be shown not to be binding of a catecholamine oxidation product nor hormone binding to a catabolic enzyme such as COMT.

Birnbaumer (23) and others (11, 24) have attempted to explain the discrepancies between the binding and activation studies by extending the model of Robison et al. (6) to include a third component, usually called a transducer, to understand the primary events in the adenyl cyclase system. This third component includes the membrane in a rather nondescript way and thus suggests that the biochemical events and membrane structural properties may be very intimately related. From catecholamine binding studies in this laboratory on several different membrane systems we have arrived at a similar hypothesis. However, rather than consider "components" of this enzyme system, we propose that there exist within the membrane distinct subunits in the adenyl cyclase system, by separating the receptors from the catalytic units and assuming they occupy separate locations within the membrane. We will show here how this hypothesis can be used to bring together the chemical and physical properties of the membrane to construct a quantitative model for the action of hormones via the cell membrane.

THEORY AND RESULTS

Using the current concepts of membrane structure (4, 25, 26), consider the membrane to be made up of amphipathic lipid molecules arranged in a bilayer with their hydrophilic heads facing the exterior and their tails interacting so as to form a hydrophobic interior of the membrane, as shown in Fig. 1. Embedded in this lipid matrix are largely hydrophobic macromolecules (i.e. mainly globular proteins, glycoproteins, and lipoprotein complexes) forming a mosaic of randomly distributed proteins in a lipid "sea" (4). About the plane formed by the hydrophobic interactions of the two lipid layers the lipid composition is asymmetrically distributed. Such an asymmetric lipid bilayer has been demonstrated for the erythrocyte membrane (25, 26). That is, of the four major phospholipids of the erythrocyte membrane, only two, phosphatidyl-serine and phosphatidyl-ethanolamine have reactive amino groups, neither of which is accessible from the exterior side of the membrane (27). Furthermore, van Deenan and co-workers were able to show that 80–85% of the sphingomyelin and 65–70% of the lecithin is located on the outer side of this membrane (28).

In a similar way, the membrane proteins also show an asymmetric distribution within the membrane. While many of the membrane glycoproteins are accessible to the exterior surface of the membrane, other hydrophobic macromolecules are located on the inner side (i. e. are not accessible from the outside). Thus, the membrane is proposed to be made up of "intrinsic" proteins (29) which are hydrophobic proteins partly surrounded by lipid, some of which are located within the external half of the bilayer protruding out

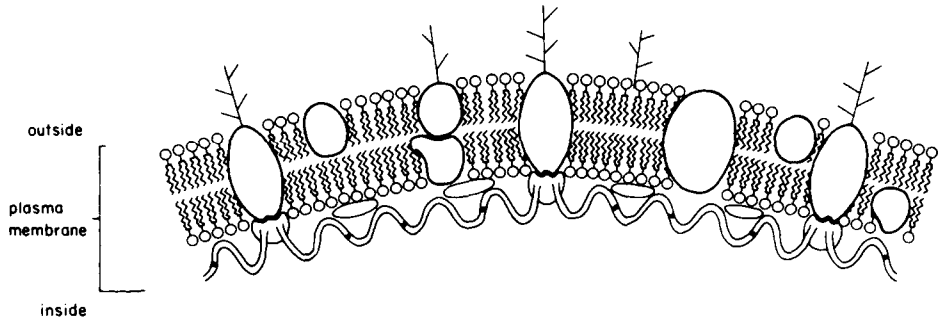

Fig. 1. A schematic disposition of the protein and phospholipid components of the plasma membrane in a cross-sectional view. Phospholipids are arranged in the membrane with their polar heads (open circles) extended outward and their fatty acid chains (zigzag lines) positioned to form an asymmetric (with respect to lipid composition, not shown) bilayer. The intrinsic proteins are represented as globular molecules (large oval shapes) embedded in the lipid bilayer, some confined to the inner side with their polar parts extending to the lumen, others on the outer side with their polar groups exposed to the extracellular medium, while others span the entire membrane. Sialic acid-containing glycoproteins are shown with the sugar residues (branched lines) protruding into the extracellular space. The extrinsic proteins are drawn on the luminal side of the membrane.

of the membrane, some situated within the internal side reaching the lumen, and others spanning the entire membrane (25–27). Some of these proteins are chemically bound to lipid as lipoproteins while some may be chemically bound to oligosaccharides as glycoproteins. Also associated with the membrane are "extrinsic" proteins (29) which are relatively more hydrophilic and water soluble. They appear mainly on the interior side of the membrane.

Lateral diffusion of the intrinsic proteins within the membrane is well documented for several different systems (24, 30–34). Diffusion occurs within the lipid matrix, in the plane of the bilayer. Such diffusion within the lipid has been observed for immunoglobulin on the surface of lymphocytes (32), glycoproteins in red blood cell membranes (31, 33) and rhodopsin in the visual receptor membranes (34). Furthermore, Devaux and McConnell (35) have shown that whereas lateral diffusion in a lipid bilayer is very rapid, diffusion between the layers is extremely slow.

Beginning with the basic model of Robison et al. (6), we assume that adenyl cyclase is made up of a two-component system. The regulatory subunit (R) or hormone receptor is located in the exterior half of the lipid bilayer exposed to the extracellular medium, and the catalytic (C) component on the inner side facing the cytoplasm. This concept of a two-component adenyl cyclase, dependent in some undefined manner on the phospholipid bilayer, has been postulated elsewhere (11, 23, 24). Our model differs from all others in the following two assumptions. First, this model assumes that the two components are distinct macromolecules which exist as separate entities in the membrane. The receptor lies in the external half of the membrane with one end exposed to the extracellular medium to bind with the hormone. The hydrophobic portion of this amphipathic macromolecule is assumed to extend into the lipid bilayer, deep enough to interact with the catalytic unit which is located on the internal half of the membrane. A schematic representation of this membrane model is shown in Fig. 2. In the inactive state (i.e. before hormone is added), the receptors do not bind with the catalytic units. When the hormone binds to the receptor, a conformational change occurs in the receptor

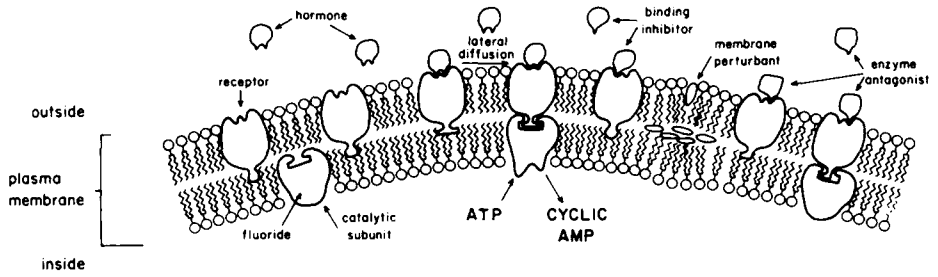

Fig. 2. The interaction of hormones with a two-component adenyl cyclase system is shown diagrammatically in this cross-sectional representation of the plasma membrane. The regulatory subunit of the adenyl cyclase system is shown as a globular protein (hormone receptor) embedded in the outer half of the lipid bilayer, geographically separated from the catalytic subunit. In the inactive state the receptor macromolecule shows very little affinity for the catalytic subunit. Hormone binding to a receptor activates it by increasing its affinity for the catalytic subunit. By lateral diffusion the two macromolecules interact and the subsequent binding activates the enzyme system. Because the membrane possesses more receptors than catalytic subunits (i.e. spare receptors), inhibitors which do not activate these receptors or change their affinity for the catalytic subunit, simply compete with hormone binding and have little influence on enzyme activity. Antagonists are shown to reduce the enzyme activity by inhibiting the binding of the activated receptor to the catalytic subunit. Membrane perturbants act by reducing the interaction between the separated subunits.

and this activated complex diffuses to the catalytic unit. The lipids are the medium in which these molecules reside and hence determine the tertiary structure of these macromolecules, the conformational changes which can occur, and the diffusion rates. Nothing more specific needs to be said about the lipid, just as, in binding studies in solution, little needs to be specified concerning the water structure.

The second assumption is that the receptor concentration [R] in the membrane greatly exceeds the number of catalytic units [C]. This is analogous to the spare receptor theory of Furchgott (36), in that it assumes that only a small fraction of the receptors need to bind hormone to induce a maximal effect.

Qualitatively, the initial events of the adenyl cyclase system are described using this model as follows (see Fig. 2). The first reaction is the binding of a ligand (L) to the membrane receptor. This binding is assumed to proceed according to the standard Langmuir isotherm. If the ligand is an agonist (A) (i.e. hormone), a change in the receptor occurs with binding (e.g., conformational change) to activate it, so that the complex RA, which is free to diffuse within the membrane, can interact with the catalytic unit. RA binding to C constitutes an activated adenyl cyclase system. Since $[R] \gg [C]$, RAC formation does not appreciably affect the equilibrium between R and A. Further, we assume that diffusion of RA to the catalytic unit is normally not a limiting step in the process. Antagonists (I) also bind to the receptor but because of the differences in their molecular structure from agonists, they produce only a partially activated receptor. This partially activated receptor is assumed to be free to diffuse to the catalytic unit and bind to it. However, the RIC complex is not enzymatically active, and hence the catalytic unit is blocked. Other ligands (N) may bind to the receptor, but are unable to activate this macromolecule and hence do not interact appreciably with the catalytic unit.

Quantitatively, this model is described by the following equations. Let [R] equal the concentration of receptors per gram of membrane; [C] equal the concentration of catalytic units per gram of membrane; and [L] equal the concentration of ligand per

liter of sample. L may be an agonist, A, antagonist, I, or a binding inhibitor, N. In a dilute solution, the binding of a hormone to a receptor in the absence of a competitive inhibitor is given by

$$[RA] = [R]/(1 + k_3/[A]), \tag{1}$$

where [RA] is the concentration of complexed receptors in the membrane, and k_3 is its dissociation constant.

In the presence of a competitive inhibitor L which also binds to the receptor R with a dissociation constant k_4, this equation becomes:

$$[RA] = [R]/\left(1 + \frac{k_3}{[A]} + \frac{k_3}{[A]}\,\frac{[L]}{k_4}\right), \tag{2}$$

and

$$[RL] = [R]/\left(1 + \frac{k_4}{[L]} + \frac{k_4}{[L]}\,\frac{[A]}{k_3}\right). \tag{3}$$

The concentration of activated receptors complexed to catalytic units in the absence of inhibitors is given by:

$$[RAC] = [C]/(1 + \frac{k_1}{[RA]}), \tag{4}$$

where k_1 is the dissociation constant for the RAC complex.

In the presence of competitive inhibitors which bind to the catalytic unit with a dissociation constant k_2, one obtains:

$$[RAC] = [C]/\left(1 + \frac{k_1}{[RA]} + \frac{k_1}{[RA]}\,\frac{[RL]}{k_2}\right). \tag{5}$$

Now consider catecholamine binding to a membrane receptor. For epinephrine binding to the receptor, we have taken from our own studies a dissociation constant of $k_3 = 10^{-6}$, which is in good agreement with other binding studies (17–20). In Fig. 3 the fraction of hormone receptor complexed is given as a function of hormone concentration, the complex [RA] being calculated from Eq. (1). Assuming the enzyme activity is determined by the amount of RAC formed, one can quantitate adenyl cyclase activation provided the dissociation constant for this complex is known. Using $k_1 = 10^{-3}$ [R], the values for this activation shown in Fig. 3 were calculated from Eq. (4). This figure demonstrates both hormone binding and enzyme activation in the absence of any other inhibitors of binding or antagonist of enzyme activity.

Using the same values for k_1 and k_3 as given above, Fig. 4 demonstrates the effect of introducing an antagonist, such as propranolol, on both hormone binding and enzyme activity. Since it is known that propranolol does not inhibit binding appreciably, k_4 was set equal to 10^{-2} in Eq. (2) to obtain the hormone-binding results shown in Fig. 4. On

the other hand, since this β-antagonist is known to inhibit efficiently adenyl cyclase activity, k_2 was set equal to 10^{-8} [R]. Figure 4 shows the insensitivity of hormone binding under these assumptions to 10^{-8}, 10^{-6}, and 10^{-3} molar propranolol. At the same time, however, the enzyme activity as determined by the formation of the RAC complex is observed to decrease by approximately 50% for 10^{-8} M inhibitor, 98% for 10^{-6} M inhibitor, and 100% for 10^{-3} M inhibitor, all in the presence of 10^{-7} M epinephrine.

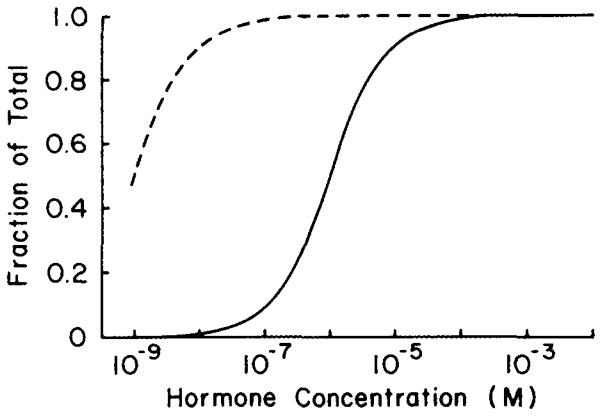

Fig. 3. Hormone binding to the membrane receptors and adenyl cyclase activity calculated in the absence of antagonists or binding inhibitors. The percent of hormone bound as a function of agonist concentration was calculated from Eq. (1) with $k_3 = 10^{-6}$ (see text), and is given by the solid (——) line. The adenyl cyclase activity is determined by the amount of activated hormone-receptor complex (RA) binding to the catalytic subunit (C). This RAC complex as shown by the dashed (- - - -) line was calculated from Eq. (4) (see text) with $k_1 = 10^{-3}$ [R].

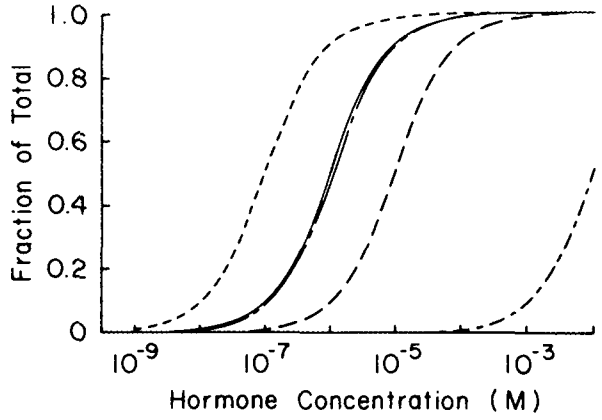

Fig. 4. The effect of an antagonist on hormone binding to the membrane receptor and on adenyl cyclase activity. Hormone binding in the presence of several different concentrations of antagonist was calculated from Eq. (2) given in the text with $k_1 = 10^{-3}$ [R] and $k_3 = 10^{-6}$ as in Fig. 3, and $k_4 = 10^{-2}$. The percent of hormone bound is shown in the presence of 10^{-8} M antagonist (——) and 10^{-4} M antagonist (— - —). The percent of hormone bound in the presence of 10^{-6} M antagonist is indistinguishable from that in the presence of 10^{-8} M antagonist. Using these results, the enzyme activity was calculated from Eq. (5) (see text) with $k_2 = 10^{-8}$ [R], in the presence of 10^{-8} M (- - -), 10^{-6} M (— — —), and 10^{-4} M (— - — - —) antagonist. See Fig. 3 for the activity in the absence of antagonists.

The third condition of interest is the effect of a nonspecific inhibitor on the adenyl cyclase system as predicted by this model. By a nonspecific inhibitor is meant a ligand which does not inhibit the enzyme activity yet competes for the receptor binding. In the epinephrine system, such a ligand might be dopamine.* From the ability of dopamine to compete for the epinephrine receptor sites (McGuire and Barber, unpublished observations), we have set, as an upper limit for this binding, $k_4 = 10^{-6}$. Using Eqs. (2) and (5), the effect of 10^{-6} M and 10^{-4} M dopamine on epinephrine binding and adenyl cyclase activity are shown in Fig. 5. In the presence of 10^{-6} M epinephrine, where in the absence of any inhibitor 50% of the receptors are in the complex RA, this model predicts that hormone binding is reduced to 33% of its maximum, and to 1% of this value by the addition of 10^{-4} M dopamine. On the other hand, since RNC is not a stable complex, the enzyme activity is not affected by the addition of 10^{-6} M dopamine, being reduced only by 9.5% in the presence of 10^{-4} M dopamine.

It should be noted that in the above examples, the values for the dissociation constants were chosen in agreement with the experimental data when possible. These values are not critical to this model, since a fairly large range of values can be shown to give similar results.

DISCUSSION

The model of adenyl cyclase as proposed here is a three-component system including the receptor, the membrane, and the catalytic unit. The membrane is considered to be an integral part of this system because: (1) it allows physical separation of the catalytic units from the receptors and yet confines them locally within the cell; (2) it gives directionality to this system by restricting the motion of the receptors to a

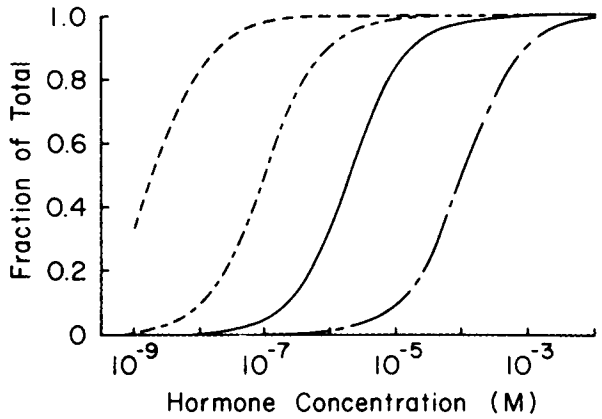

Fig. 5. Hormone binding and enzyme activity as determined in the presence of a nonspecific inhibitor (i.e. a ligand which competes for hormone binding). Using $k_1 = 10^{-3}$ [R] and $k_3 = 10^{-6}$ as in Fig. 3, k_4 was set equal to 10^{-6} in Eq. (2) (see text) to calculate the percent of hormone bound as a function of agonist concentration. The results are shown for binding in the presence of 10^{-6} M (——) and 10^{-4} M (— · —) inhibitor. The adenyl cyclase activity as determined by the formation of RAC was calculated from Eq. (4) (see text) for 10^{-6} M (- - -) and 10^{-4} M (– – – –) inhibitor.

*In fact, dopamine has been shown to act as an agonist in several systems (37), however, it is more commonly found to act as neither an agonist or antagonist.

plane; and finally (3), it is the milieu within which the activated receptors must diffuse to interact with the catalytic units. The receptors are protein components of the cell membrane, which are hormone specific (6, 38) and, although there are more receptor macromolecules than catalytic units, each receptor is potentially a functional part of the adenyl cyclase system. Since these molecules are assumed to exist in the membrane separated from the catalytic units, geometrical considerations are not important in allowing numerous receptors to interact with the same catalytic unit.

In this model, although hormone binding to a specific membrane receptor is necessary, the mechanism of adenyl cyclase activation is not direct and hence neither the binding concentration curve nor the kinetics of binding are necessarily related in a straightforward way to enzyme activation. Thus, as demonstrated by Fig. 3, maximal enzyme activation is possible at low concentrations of hormone binding. Similarly, using a constant hormone concentration, short times of incubation which do not give high percentages of hormone bound can in fact give sufficient binding to yield maximal activation.

In a similar way, this model may be used to explain the apparent discrepancies in catecholamine binding and activation of adenyl cyclase. Although many questions concerning catecholamine binding have been raised, careful experiments have shown that this binding is both saturable and reversible. The main problems are the lack of stereospecificity, the ability of compounds with a 3, 4-dihydroxyphenyl moiety to inhibit binding but not serve as antagonists, and the inability of known β-antagonists to inhibit binding. Most catecholamine-binding studies do show specificity for the alkyl side chain (15–19), and the order of the binding agrees with that predicted as an α- or β-type receptor. In addition, work from this laboratory on a catecholamine-binding macromolecule has shown that a conformational change is highly dependent on the alkyl side chain (McGuire and Barber, manuscript in preparation). These observations, together with the known role of the hydrophobic and hydrophilic interactions in determining the double helix structure of deoxyribonucleic acid (DNA) (i.e. the main energy of formation of DNA comes from the relatively nonspecific hydrophobic interactions or base stacking while the specificity is determined through the formation of hydrogen bonds), suggests a similar type of interaction between the catecholamines and membrane receptors. That is, if the catechol moiety interacts with the receptor through hydrophobic bonding, this would be the main contribution to the overall stability of this complex and hence determine the dissociation constant. On the other hand, the alkyl side chain would dictate the specificity of the reaction (i.e. adenyl cyclase activity) through the formation of hydrogen bonds. Since hydrogen bonds with the solvent (water) must be broken before new ones can be formed, they contribute relatively little to the stability of the complex, while still being crucial to the formation of an activated receptor (e.g., by causing a conformational change). As shown in Fig. 5, this model predicts that nonspecific inhibitors (here the d-isomer is assumed to belong to this class) should inhibit binding yet have little effect on the enzyme activity, especially in the presence of high (10^{-6} M) concentrations (relative to the number of catalytic units) of agonist. On the other hand, β-antagonists which do not contain a catechol group would have little effect on binding, and yet be antagonistic to the biochemical reaction (see Fig. 4).

Recently, a number of studies have supported the initial observation of Sutherland and co-workers that many perturbants of membrane structure have grave consequences on the adenyl cyclase system. Sutherland et al (39) were the first to observe that nonionic

detergents (which should interact primarily with the lipids in the membrane) abolish hormone responsiveness. Lubrol-PX and Triton X-100 have both been shown to cause partial or complete loss of hormone sensitivity but have little effect on the NaF-sensitive activity. Phospholipase A has been shown to enhance binding of glucagon, TSH, and ACTH, yet in all of these systems it inhibits the adenyl cyclase responsiveness to these same hormones. Birnbaumer et al. (40) demonstrated that the adenyl cyclase response in the fat cell ghost to epinephrine, ACTH, secretin, and glucagon but not NaF was abolished by treatment with digitonin.

More recently, Wolff and Jones (41), using chlorpromazine as well as several other phenothiazenes, demonstrated that the adenyl cyclase response to thyrotropin and prostaglandin in thyroid membranes, ACTH stimulation in the adrenal tissue, and glucagon and epinephrine stimulation in the liver are inhibited. At the same time NaF-stimulated cyclase in the thyroid and adrenal tissue was increased by these agents. They concluded that these agents interrupted the hormone effect on the cyclase activity by altering the membrane structure. Similar results have been obtained using high concentrations of propranolol and phentolamine by Levey et al. (42) and by Lang et al. (14) using phenoxazones.

Consequently, this adenyl cyclase system is extremely sensitive to its environment (namely the membrane structure) which can drastically alter the hormone-receptor-catalytic subunit relationships. Hence, as is quantitatively demonstrated by this model, while the hormone-receptor interaction is a necessary condition for enzyme activity, it is not simultaneously a sufficient condition for hormone action. The complexity of this system requires the simultaneous control of numerous factors. Therefore, while this model, or any model, cannot prove or disprove the system, hopefully its description offers new insights into this complex enzyme and hence indicates new experimental approaches to delineate one mechanism of hormone action at the membrane level.

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