# Plasma Membrane Receptors

ROBERT J. LEFKOWITZ and THOMAS MICHEL, *Howard Hughes Medical Institute, Departments of Medicine (Cardiology) and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710*

Receptors are macromolecules in or on cells that mediate physiological responses upon binding specific ligands. Over the past few years, important advances have been made in the understanding of the biology of receptors, both at a basic and at a clinical level. Receptors bind their ligands with remarkable selectivity and with high affinity, permitting a given physiological response to be elicited with great specificity in the target tissue. The specific binding of agonists to receptors leads to the generation of signals that influence cell functions. The signal may be expressed as an altered rate of enzyme activity or ion transport, which then leads to the characteristic physiological responses within the cell.

A wide variety of molecules, including hormones, drugs, neurotransmitters, growth factors, chemotactic agents, antigens, viruses, plasma lipoproteins, and glycoproteins, elicit their biological effects by virtue of their binding to cellular receptors. The more lipid soluble of these agents, such as steroid and thyroid hormones, are able to diffuse through the cell's plasma membrane and bind to intracellular receptors. Many of these agents, however, interact with receptors exposed on the external surface of the cell imbedded in the cellular plasma membrane. It is the purpose of this short essay to assess the current status of research on these membrane-bound receptors.

It is only within the past fifteen years that the most proximal event in receptor function, that of ligand binding, has become amenable to direct experimental investigation. Such ligand-binding studies have facilitated rapid advances in the study of receptors. Several membrane receptors have been purified and an understanding of their function at a molecular level is now being realized. Ligand-binding studies have also shown that the numbers and properties of receptors are dynamically modulated by a variety of factors, including disease states and by chronic exposure to hormones and drugs (desensitization). This has provided an important approach to understanding the molecular basis for control of drug, neurotransmitter, and hormone sensitivity. Furthermore, the discovery of diverse receptors on circulating cells has provided a direct approach to the study of these receptors in disease states in man.

## *Measurement of receptor binding*

Radioactive analogues of hormones and drugs may be used to label binding sites on cells and in cell-membrane preparations. In each instance it is necessary to demonstrate rigorously that the binding measured reflects association of the ligand with the relevant receptor. Many sites other than receptors may bind radioactive ligands saturably, with high affinity and with definable specificity. The most important hallmark of a receptor binding site identified in a radioligand binding assay is its detailed specificity. This specificity should be virtually identical to the specificity of the physiological response that is mediated by that receptor. When stereospecificity characterizes the ability of drugs or hormones to elicit a response, it should also be expressed in the results of binding studies.

The amount of radioligand bound in a particular cell or membrane preparation is a function of the re-

*Received for publication 14 July 1983.*

ceptor concentration, its affinity for the radioligand, and the concentration of the radioligand. Therefore, the determination of the amount of a radioactive ligand bound to a cell or membrane preparation at a single concentration of radioligand, a practice often encountered in the literature, provides little information about the status of the receptors and does not even distinguish between a change in receptor affinity for binding ligands and a change in the number of receptors.

Recently, there has been a lively debate in the literature as to the best way to analyze ligand-binding data. Klotz (1) contends that the commonly used Scatchard plot ($B/F$ vs. $B$, where $B$ and $F$ refer to the amounts of bound and free ligand, respectively, over a range of free ligand concentrations)[1] can provide misleading values for the number of receptor sites. He prefers a graph of $B$ vs. $F$ with $F$ displayed on a log scale with a wide enough range of concentrations to make certain that measured $B$ values exceed $\frac{1}{2}B_{max}$. However, this approach is impractical in many biological systems because direct radioligand binding is confined to a narrow concentration range because of increased nonspecific binding at high ligand concentrations. On the other hand, indirect binding studies, e.g., binding-competition curves, are routinely plotted as log [competitor] vs. amount of radioligand bound. There are also problems with the widely used Scatchard representation: First, do points that deviate from a straight line represent errors in analysis or do they reflect some kind of binding cooperativity? Second, is the interdependence of the coordinates (bound ligand appears in both ordinate and abscissa) influencing the plot by amplifying errors in the measurements?

Rodbard and Munson (2) have emphasized that the various linearized plots referred to above, e.g., $B/F$ vs. $B$ and others such as $1/B$ vs. $1/F$, (double reciprocal) or $B$ vs. $B/F$ are all algebraically equivalent and no one plot is vastly superior. All are simply derived from the law of mass action for the reversible binding of a ligand (L) to a receptor (R) according to a bimolecular reaction: $L + R \rightleftharpoons LR$. $B$ refers to [LR], whereas $F$ refers to [L].

A powerful analytic approach is to use one of several computer-assisted nonlinear least squares curve fitting programs that directly apply the law of mass action to analysis of binding data (3, 4). This method ensures a much greater degree of accuracy in the determination of dissociation constants and number of receptors, and permits objective statistical testing of goodness of fit. It can compare alternative models (e.g., one vs. multiple classes of binding sites) with selection of the "best model" based on objective statistical criteria. The direct computer-assisted statistical analysis of binding data also avoids biases and artifacts resulting from data transformations required in most graphical methods of analysis. It provides measures of the standard errors of all estimated parameters, permits simultaneous analysis of several curves, and provides the ability to statistically compare differences between binding isotherms.

## *General insights about membrane receptors from ligand binding studies*

*The number of receptors at the cell surface is not fixed.* Receptor number is dynamically regulated, both by hormones and drugs that ordinarily combine with the receptors, as well as by other factors. For example, the number of β-adrenergic receptors on cells is regulated both by their natural ligands (catecholamines) as well as by other hormones such as thyroxine, glucocorticoids, and sex steroids (5). Such regulation of receptor number in turn often controls cellular sensitivity to hormone or drug action. The most thoroughly studied such form of receptor regulation is the regulation of receptor number by ligands referred to as "down regulation." Initially described for the insulin receptors (6) and β-adrenergic receptors (7), this phenomenon has now been documented for many plasma membrane receptors (8–10).

Several different cellular mechanisms are involved in these processes. All have in common that they are promoted by agonists but not antagonists and ultimately lead to the translocation of the surface receptors to an intracellular location. In some cases the intracellular transport is accomplished by special endocytotic vesicles that arise at the cell surface (11). Once within the cell the receptors may be degraded, processed, or recycled back to the cell surface. Agonist-promoted internalization of receptors may serve a number of very distinct functions. In some cases it serves to attenuate cellular sensitivity to further hormonal or agonist stimulation. This may be accomplished by reduction of the number of functional receptors at the cell surface and by their sequestration within the cell in compartments physically removed from the normal effector units. This is one of a number of mechanisms responsible for desensitization or tachphylaxis to drug and hormone actions. Alternatively, internalization of the hormone-receptor complex may serve as an essential pathway in the activation of the biological response. The study of the mechanisms

[1] *Abbreviations used in this paper:* $B$, amount of bound ligand; $F$, amount of free ligand.

whereby ligand occupancy of receptors triggers their internalization remains an active area of research.

*Receptor-binding studies have helped to illuminate the molecular mechanisms of drug and hormone action.* That receptors both bind ligands and activate biological processes is hardly a new notion. However, it had earlier been thought that only the first of these functions could be probed by ligand-binding approaches. In fact, significant insights concerning the second or "activating" function of receptors may also be obtained from such studies. One particularly fruitful approach has been the investigation and comparison of the binding characteristics of agonists and antagonists. Agonists and antagonists both bind to the same receptor, but, since agonists promote biological effects, whereas antagonists do not, it was reasoned that differences in their binding properties might provide clues as to the mechanistic basis for these differences in their effects.

The most thoroughly studied system has been the adenylate cyclase-coupled $\beta$-adrenergic receptor. Such systems consist of three distinct molecular components (Fig. 1). These are the hormone receptor (R), the catalytic moiety of the adenylate cyclase (AC), which converts ATP to cyclic AMP (cAMP), and a coupling protein (N) that is regulated by guanine nucleotides such as GTP. This latter protein is involved in "coupling" receptor binding of agonist to activation of the enzyme. Using nonlinear least-squares computer analysis of agonist-binding competition curves, it was found that the receptors are capable of existing in two discrete states having either low or high affinity for an agonist (12). Biochemical studies subsequently indicated that these two forms of the receptor corresponded to R (low affinity) and RN (high affinity), and that the equilibrium between the states of the receptor

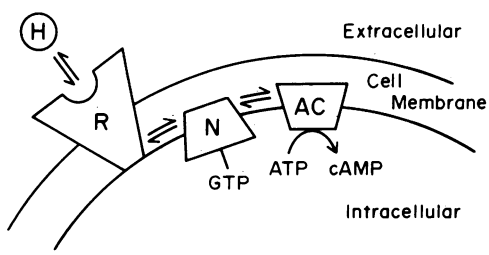


FIGURE 1 Schematic diagram of the components of hormone-responsive adenylate cyclase systems such as that coupled to the $\beta$-adrenergic receptor.

is described by the equation: $R + N \rightleftharpoons RN$ (12). Guanine nucleotides such as GTP bind to the coupling protein and are required for activation of adenylate cyclase by hormones. Agonists promote the interaction of hormone receptor with coupling protein, which leads to adenylate cyclase activation. Antagonists do not favor formation of this complex. Since antagonists display equal affinity for the two states of the receptor, their competition curves are uniphasic and conform quite closely to what is predicted by the law of mass action for binding to a single class of sites. In contrast, agonist competition curves are biphasic but are shifted to lower affinity and become uniphasic in the presence of guanine nucleotides. Guanine nucleotides convert all the high affinity state receptors into low affinity state receptors coincident with hormone-promoted activation of the enzyme (12). This phenomenon has been demonstrated for a wide variety of hormone, drug, and neurotransmitter receptors, which are coupled to the effector adenylate cyclase and even for some receptors where coupling to the adenylate cyclase system has not been demonstrated. An example of the latter is the receptor for chemotactic peptides on polymorphonuclear leukocytes (13).

There are many other more and less complicated models of ligand receptor interaction. These models include allosteric or "two-state" models, various cooperative models, and divalent receptor models. In summary, ligand binding data, when carefully analyzed, can help to sort out the underlying molecular mechanisms involved and suggest further, more definitive, biochemical experiments.

In clinical investigation, a detailed understanding of normal biochemistry and physiology is often rapidly translated into increased knowledge about how various pathophysiologic circumstances alter normal processes. Since ligand-binding data may reflect molecular interactions between receptors and other components of the effector system, such data can be used to assess the status of such interactions in various physiologic, therapeutic, and pathophysiologic circumstances. For example, recent findings indicate that formation of the agonist-$\beta$-adrenergic receptor-nucleotide regulatory protein complex is modulated by a variety of physiological and pathophysiological circumstances and that such modulation represents an important mechanism for the control of tissue sensitivity to catecholamine action (5). Several clinically relevant conditions are associated with an attenuation of the ability of $\beta$-adrenergic agonists to stimulate adenylate cyclase and include such diverse conditions as desensitization due to prolonged exposure to agonist, hypothyroidism, hypoadrenalism, and pseudohypoparathyroidism type 1A. In each case the altered receptor responsiveness

can be documented directly in agonist-binding competition curves, and is apparent as a shift in the competition curve to a lower affinity. The change in affinity is because of a decreased stability of the high affinity receptor complex. Thus, these findings suggest that the "uncoupling" observed is due to impaired interaction of hormone receptor and coupling protein to form the obligatory intermediate complex containing the agonist, the β-adrenergic receptor, and the nucleotide regulatory protein.

Modulation of the interaction between hormone receptor and coupling protein may represent a fundamentally important point of regulation in the control of tissue sensitivity to β-adrenergic stimulation (5, 12). In this particular example, the effector is an enzyme, adenylate cyclase, whereas in other cases it might be an ion channel or transport protein. These findings also emphasize that both receptor number and receptor coupling are dynamically regulated. The mechanisms by which coupling of receptors to effectors, such as the coupling protein of the adenylate cyclase system, are regulated are just beginning to be elucidated. For example, recent work has suggested that covalent modification of β-adrenergic receptors may be one mechanism leading to the receptor "uncoupling" that accompanies some forms of agonist-promoted desensitization (12).

### *Molecular characterization of membrane receptors*

There have been two major experimental approaches to the molecular characterization of membrane receptors: purification of the receptors and identification of the receptors with ligands that bind irreversibly. Most cell membrane receptors are present in minute quantities and require up to 100,000-fold purification to obtain homogeneous preparations. The membrane proteins must be solubilized with detergents before purification efforts can even begin, and the solubilized receptors must possess the essential binding specificity characteristics of the membrane-bound receptors. Several methodological advances have greatly accelerated progress in this area. One is affinity chromatography, a technique wherein a ligand that binds to the receptor is immobilized on a solid support (14). The solubilized receptor preparation is then applied to a column containing the immobilized ligand. The receptors may be biospecifically adsorbed, whereas most of the other proteins pass through. The receptors can then be biospecifically eluted with any ligand, such as a hormone or drug, that binds to the receptors and competes for binding with the immobilized ligand. Such procedures generally yield appreciable purification of membrane receptors, although additional steps are usually necessary to achieve complete purification.

The other technique that has recently greatly facilitated elucidation of the structure of membrane receptors is photoaffinity labeling (15). This labeling can be accomplished by the synthesis of hormone and drug analogues that contain a photoactive moiety, such as an azide group. Once such a ligand is bound to the receptors in particulate or soluble preparations, ultraviolet irradiation generates a reactive species (e.g., a nitrene) that leads to the covalent incorporation of the ligand into receptor proteins. Since many such ligands can be radioactively labeled, the size of the receptor can be assessed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate and autoradiography of the gel to identify the receptor-ligand complex. Assurance that such irreversible ligands are actually bound to the receptors is obtained by documenting that other agents that combine with the receptors appropriately block incorporation of the radiolabeled irreversible ligand. Using such techniques, a variety of membrane receptors have been identified. All appear to be integral membrane glycoproteins with apparent molecular weights ranging from 30,000 to 300,000 (12, 16–18). In some cases, the ligand-binding site resides on a single peptide chain that comprises the active receptor, as with the β-adrenergic receptor (12). In other cases, the binding site resides on a peptide that is noncovalently bound to other protein subunits (which presumably subserve an effector function) in an oligomeric structure. The nicotinic cholinergic receptor is an example of this type of receptor (17). For other receptors the ligand binds to a peptide that is covalently bound to distinct peptide chains that may bear an effector activity, e.g., a protein kinase. The insulin receptor provides an example of this structural arrangement (19).

### *Future prospects*

Ligand-binding techniques for the study of membrane-bound receptors are now used in virtually every branch of biomedical science. Proper application of the methodology and judicious interpretation of the data generated can provide insights well beyond the simple enumeration of the number or affinity of receptors in tissues under varying conditions. The ability to solubilize and purify the receptors has opened the way to detailed studies of their molecular structure and the functional consequences of their modification in various pathophysiological circumstances. It is reasonable to predict that within the next few years the various effectors of these receptors will also be purified and reconstituted with the isolated receptors. Such re-

constituted systems should provide important insights into mechanisms of physiological regulation of receptor function at the molecular level.

## REFERENCES


1. Klotz, I. M. 1982. *Science (Wash. DC).* 217:1247–1249.
2. Munson, P. J., and D. Rodbard. 1983. *Science (Wash. DC).* 220:979–981.
3. Munson, P. J., and D. Rodbard. 1980. *Anal. Biochem.* 107:220–239.
4. De Lean, A., A. Hancock, and R. J. Lefkowitz. 1982. *Mol. Pharmacol.* 21:5–16.
5. Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. 1983. *Physiol. Rev.* In press.
6. Gavin, J. R., III, J. Roth, D. M. Neville, Jr., P. DeMeyts, and D. N. Buell. 1974. *Proc. Natl. Acad. Sci. USA.* 71:84–88.
7. Mukherjee, C., M. G. Caron, and R. J. Lefkowitz. 1975. *Proc. Natl. Acad. Sci. USA.* 72:1945–1949.
8. Schwall, R. H., and G. F. Erickson. 1983. *J. Biol. Chem.* 258:3442–3445.
9. King, A. C., L. Hernaez-Davis, and P. Cuatrecasas. 1980. *Proc. Natl. Acad. Sci. USA.* 77:3283–3287.
10. Pastan, I. H., and M. G. Willingham. 1981. *Annu. Rev. Physiol.* 43:239–250.
11. Goldstein, J. L., R. G. W. Anderson, and M. S. Brown. 1979. *Nature (Lond.).* 279:679–685.
12. Lefkowitz, R. J., J. M. Stadel, and M. G. Caron. 1983. *Annu. Rev. Biochem.* 52:159–186.
13. Koo, C., R. J. Lefkowitz, and R. Snyderman. 1983. *J. Clin. Invest.* 72:748–753.
14. Lowe, C. R. 1979. An introduction to affinity chromatography in laboratory techniques. *In* Biochemistry and Molecular Biology. T. S. Work, and E. Work, editors. Elsevier/North Holland, New York. 274–518.
15. Chowdhry, V., and F. H. Westheimer. 1979. *Annu. Rev. Biochem.* 48:293–325.
16. Massague, J., P. F. Pilch, and M. P. Czech. 1980. *Proc. Natl. Acad. Sci. USA.* 77:7137–7141.
17. Conti-Tronconi, B. M., and M. A. Raftery. 1982. *Annu. Rev. Biochem.* 51:491–530.
18. Metsikko, M. K., and H. J. Rajaniemi. 1982. *Biochem. J.* 208:309–316.
19. Kasuga, M., F. A. Karlsson, and C. R. Kahn. 1982. *Science (Wash. DC).* 215:185–187.