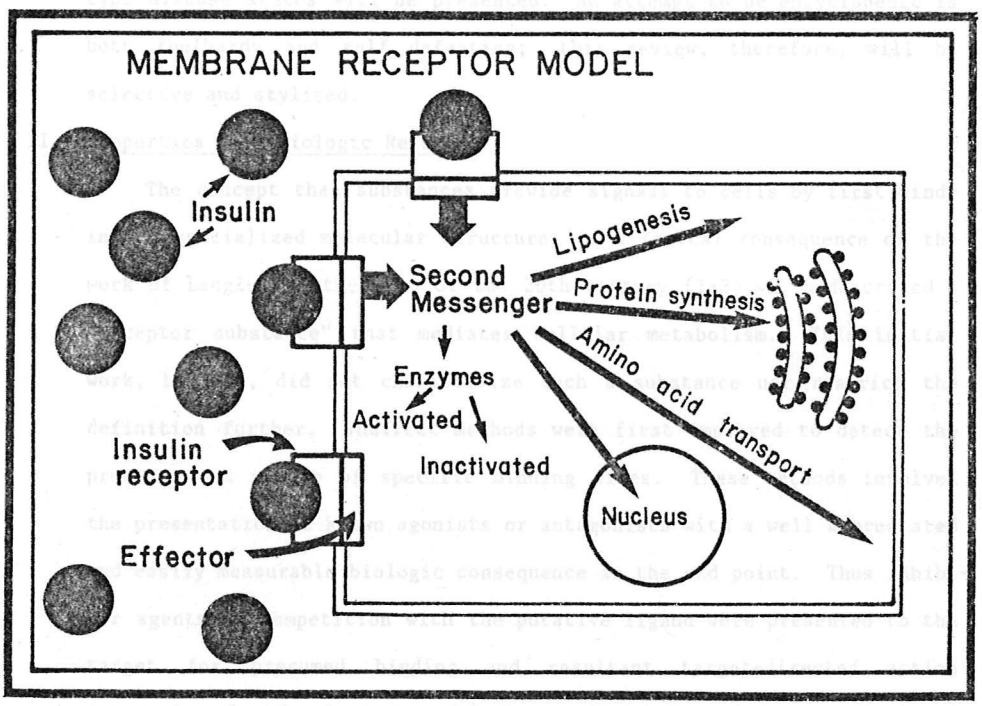
Receptors: A Mechanistic Approach to Selected Diseases

Medical Grand Rounds August 13,1981

J. Harold Helderman



University of Texas
Health Science Center at Dallas

I. Introduction

Receptors are molecules which receive and pass signals from circulating quanta of information to the cell. The growing understanding of receptor function and chemistry has profoundly altered perception of normal physiology and disease pathogenesis. The purpose of this review is to familiarize the reader with conventional receptor nomenclature, to describe basic mechanisms which govern receptor function, and to demonstrate a relationship between deviant receptor function and disease. A schema of receptor related defects will be constructed for which prototype disease states will be presented. An attempt to be encyclopedic is both foolhardy and self defeating; this review, therefore, will be selective and stylized.

II. Properties of a Biologic Receptor

The concept that substances provide signals to cells by first binding to specialized molecular structures is a logical consequence of the work of Langley at the turn of the 20th century (1-3) which described a "receptor substance" that mediates cellular metabolism. This initial work, however, did not characterize such a substance nor restrict the definition further. Indirect methods were first employed to detect the presence and nature of specific binding sites. These methods involved the presentation of known agonists or antagonists with a well appreciated and easily measurable biologic consequence as the end point. Thus inhibitor agents in competition with the putative ligand were presented to the target for presumed binding and resultant target-directed action measured. In the last two decades, radiolabel techniques, developed independently in the laboratories of Jensen, Roth and Cuatrecasas, permitted direct measurement of specific receptors.

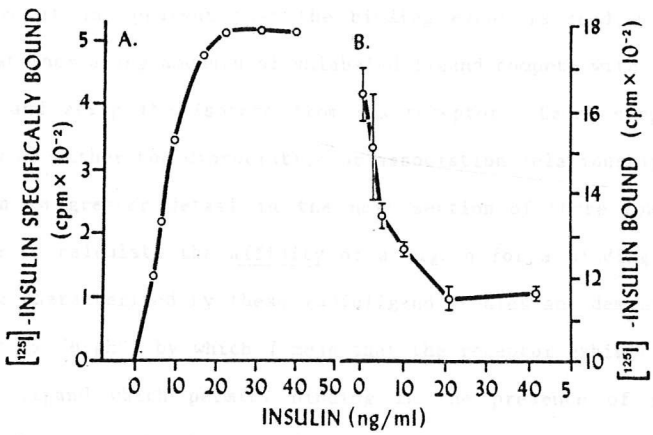
Using the events that transpire during the ligand-receptor binding event, one can generate a set of criteria for the characterization of a biologic receptor. A binding event should exhibit five properties for one to define directly a biologic receptor as listed in Table 1.

Table 1

1. reversibility
2. saturability
3. "high" affinity
4. specificity
5. biologic function induced after binding with a physiologic concentration of ligand.

Typically one performs a binding study by incubating to equilibrium conditions radiolabeled ligand with a cellular preparation (whole cells, membrane fractions, affinity column purified, soluble receptor) thought to contain a biologic receptor, followed by separation of the receptor bound ligand from the reaction mixture in which free hormone resides. In one technique called an association binding study, increasing concentrations of isotopic ligand are provided in a fixed amount to a receptor preparation as shown in the left panel of Figure 1.

Figure 1 - Association and dissociation binding studies

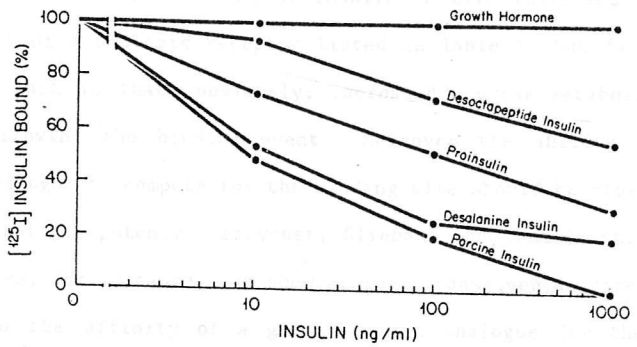


In a second technique a single concentration of the tracer ligand is provided to a receptor preparation for binding followed by the presentation of an increasing amount of unlabeled material, a dissociation binding curve (right panel, Figure 1). The association and dissociation binding curves depicted in Figure 1 illustrate the manner in which radiolabel studies give support for the presence of a biologic receptor. This figure, like many in this grand rounds, is drawn from data derived in my laboratory and thus examines the insulin receptor system on lymphocytes but is really presented to be illustrative of the more general principles I am discussing. Note that as one provides an increasing amount of the ligand to the fixed amount of material felt to contain receptor, a concen-

tration is reached above which one observes no further specific binding, the preparation exhibits saturability. Examining the dissociation binding curve it is apparent that the binding event is readily reversible such that increasing amounts of unlabeled ligand compete with the labeled species and strip the isotope from its receptor. Using simple kinetic analysis of either the dissociation or association relationships, as will be shown in greater detail in the next section of these rounds, it is possible to calculate the affinity of a ligand for a binding site which is being characterized by these radioligand studies and demonstrate that affinity is "high", by which I mean that the receptor exhibits an affinity for ligand which permits binding in the presence of physiologic concentrations of the ligand. For the studies shown in Figure 1, for example, affinity of insulin for the lymphocyte insulin receptor turned out to be 1 nM a physiologically relevant value.

Another important property of a biologic receptor is that the system exhibits specificity, the ability to recognize and bind only a limited number of molecules with highly defined structure. The series of dissociation binding curves shown in Figure 2 for the lymphocyte insulin receptor system exhibits the kind of molecular specificity required to characterize biologic receptors.

Figure 2 - Displacement of trace insulin from its receptor by insulin analogues



The unmodified porcine insulin is most effective at displacing tracer porcine insulin, while the unrelated ligand, growth hormone, is unable to displace isotopic insulin. The relative affinity of binding of several insulin analogues depends greatly on the degree to which the charge, structure, and/or spatial configuration of the molecule is altered. In this example removal of a single alanine from insulin alters receptor recognition only slightly, while removal of 8 specific amino acids from the insulin B chain decreases greatly the ability of the desoctapeptide analogue to displace unmodified tracer hormone.

Performing studies which reveal a binding event that fulfills the criteria of reversibility, saturability, and specificity is inadequate alone to characterize a biologic receptor. One must further demonstrate that a biologic consequence flows directly as a result of the binding event. This important point was emphasized by Hollenberg and Cuatrecasas when they showed that binding of insulin to talc fulfilled the first 4 properties of a biologic receptor listed in Table 1, but failed to fulfill the last in that, obviously, increased glucose metabolism did not occur following the binding event. Moreover the ability of a given ligand analogue to compete for the binding site should be closely related to its biologic potency. Freychet, Gliemann and Gammeltoft, Hollenberg and our lab, for instance, can show biologic consequences directly proportional to the affinity of a given insulin analogue for the receptor. Each year the presence of many new receptors is heralded in the scientific literature in which the demonstration of biologic meaning to the binding event is not included. The reader of such studies needs to be cautious about the acceptance of such claims until this fifth and crucial receptor property is described. The biologic event that follows binding should be measurable at concentrations of the ligand which are physiologically and kinetically relevant. Unless there are unique problems with a given in vitro assay, the use of pharmacologic amounts of ligand to produce a biologic event is suspect.

III. Kinetics of Binding

The goal of this section is to discuss the origin and meaning of much of the nomenclature used in receptor chemistry and physiology.

Table 2 is a glossary of useful receptor terminology adopted from that of Pollet for the reference of the reader.

Table 2. Frequently Used Receptor Terminology

Ligands	Relatively small molecules, that bind to larger molecules.
Specific binding	Saturable binding of radiolabeled hormone that is displaceable by excess unlabeled hormone. This functional definition differentiates true receptor sites from other non-biologic binding sites.
Scatchard plots	A graph of specific binding data in which bound/free ligand (ordinate) is plotted as a function of bound-ligand (abscissa).
Spare receptors	A large number of identical potentially functional receptors, in excess of that occupied during the generation of a maximal hormone response.
Down regulation	A homeostatic mechanism in which there is a decrease in the number of specific hormone receptors in response to elevated levels of that hormone.
Up regulation	A homeostatic mechanism in which there is an increase in the number of specific hormone receptors in response to depressed levels of that hormone or to elevated levels of a trophic hormone.
Negative cooperativity	A proposed mechanism in which the binding of a hormone causes a decrease in the binding affinity of ligand for the remaining receptor sites.
Hormone resistance	The production of a diminished hormonal effect by a given concentration of bioactive hormone, resulting from a decreased hormone sensitivity, decreased maximal hormonal response, or a combination of both.
Association binding study	Provision of increasing concentrations of labeled hormone to receptor bearing tissue for binding.
Dissociation binding study	Provision of trace labeled hormone and increasing concentrations of cold hormone to remove bound label.
Affinity	Property of a ligand related to how avid that ligand is bound to a given receptor or a given state of the receptor. Can be obtained from the slope of a Scatchard plot.
Number of receptors	The number of binding sites fulfilling the kinetic definition of a receptor. It can be obtained from the X intercept of a Scatchard plot.

The interaction between a ligand and its receptor is a dynamic event with molecules constantly moving on to and off the binding site. These dynamic events may be described using mathematical language. In order to understand standard receptor nomenclature and later the manner in which given defects in receptor binding physiology are described, I would like to review this mathematical kinetic analysis briefly.

In the simplest terms, a receptor (R) and a hormone (H) bind and unbind at definable rates or

$$H + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [HR] \qquad [1]$$

At equilibrium, following the rules of mass action, the equation of the binding reaction becomes

$$K_a = \frac{[RH]}{[H]\ [R]} = \frac{k_1}{k_{-1}} \qquad [2]$$

More precisely the R term in the denominator represents the free receptor sites. If (RT) be total receptor sites present and (RH) those occupied, one can write the equilibrium binding equation:

$$K_a = \frac{[HR]}{[H]\ [RT-RH]} \qquad [3]$$

By convention one may describe the terms of this equation in the following manner:

$$K_a = \frac{[\text{Bound hormone}]}{[\text{free}]\ [\text{unoccupied sites}]} \qquad [4]$$

or

$$= \frac{[B]}{[F]\ [RT-B]}$$

Rearranging the terms of equation [4] one can write

$$\frac{B}{F} = (K_a RT) - (K_a)\ (B) \qquad [5]$$

which is a form of the equation for a straight line:

$$Y = b - mX$$

Subjecting the data derived from the curvilinear binding plot shown in Figure 1 to just such a mathematical transformation, one can often render the binding information as a straight line. The bound to free ratio (B/F) as a function of bound ligand (B) is called the Scatchard plot, the common convention used to describe equilibrium binding in this scientific field. From the plot or equation the actual binding data can be used to precisely define the kinetic characteristics of a given receptor. The negative slope of the Scatchard relationship is the equilibrium binding affinity constant (K_a). The X intercept value provides the maximum amount of ligand bound, which is obviously related to the total number of receptor molecules present (RT). The intercept on the X axis is taken at the point the Y axis (B/F) is 0. Equation 5 then yields:

$$(K_a)\ (B) = (K_a)\ (RT) \qquad [6]$$

Rearranging

$$B = \frac{K_a RT}{(K_a)}$$

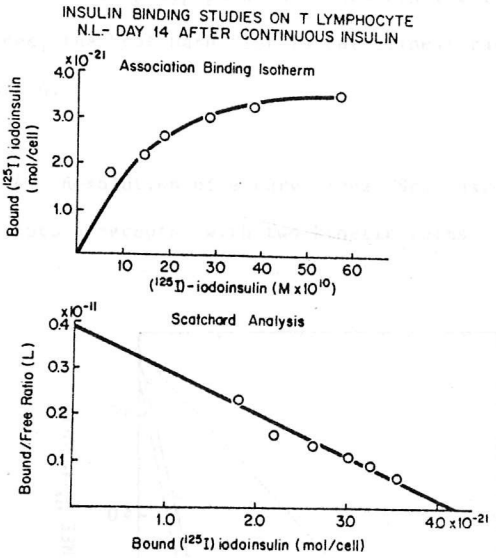
or

$$B = RT$$

which is mathematical proof of the fact that the X intercept gives the bound ligand representing total number of receptors. From this discussion it becomes clear, then, that receptor systems are defined by two kinetic properties, the equilibrium affinity constant, K_a, and the number of receptor sites present on a given cell or membrane. This review will use the concepts of receptor affinity and receptor number often during the discussions of prototypic receptor related diseases.

An example of binding data displayed as an association binding curve and then as a Scatchard plot is shown in Figure 3.

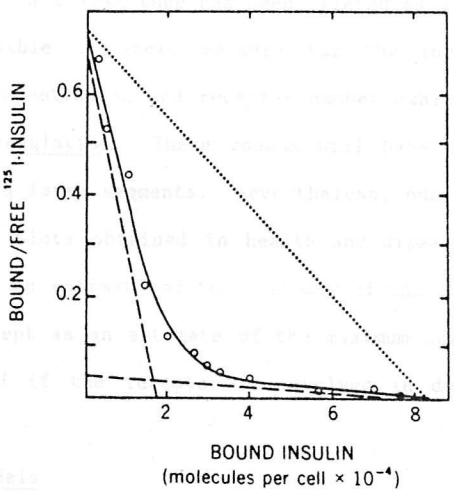
Figure 3



The association plot in the upper panel represents the familiar saturation curve discussed above. In the bottom panel the B/F ratio is plotted as a function of the Bound hormone. For this simple system of one species of receptor each with the same ligand affinity, a linear relationship results. Since the negative slope provides the affinity of the ligand for the receptor, one can inspect several such plots performed from normal or abnormal sources to determine whether an alteration in the receptor has occurred. The steeper the slope of the Scatchard line, the greater the affinity of ligand for receptor. Similarily since the X axis provides the total number of potential binding sites, inspection of the Scatchard plot can directly provide an excellent estimate of receptor

number related to the condition studied. Interpretation of defective receptor physiology from the Scatchard plot, unfortunately, is generally more complex. For most polypeptide hormones binding to a wide variety of receptor sources, the Scatchard plot is curvilinear rather than linear as shown in Figure 4.

Figure 4 - Resolution of a curvilinear Scatchard plot into a receptor with two kinetic forms



The simplest explanation of such a curvilinear plot is that there are more than a single species of binding sites. One then resolves the curvilinear plot into several, here two, straight lines. One line has a gentle slope and a large X intercept, reflecting a binding site of low affinity with high capacity; the other has a steep slope and a smaller X intercept reflecting a site of high affinity but limited capacity. Recently De Meyts provided experimental evidence, not fully accepted by all in the field, that the curvilinear

Scatchard plot is the result of progressive binding by a ligand to a single species of receptor which molecularly or spatially is in flux during the binding event so that affinity of ligand is inconstant. Receptors were said to exhibit site-site interaction of the negative cooperativity type. In simple terms this is meant that each given binding event reduces the chance of a binding event at the adjacent or neighboring unoccupied receptor. The more hormone that is bound the less chance for increased binding. Thus there is an ever decreasing affinity for a given site leading to a curvilinear Scatchard. This phenomenon which might occur in a test tube has been offered as a possible cellular mechanism responsible in great measure for the inverse relationship between ligand concentration and receptor number exhibited by many hormones, for down regulation. These rounds will have more to say about down regulation in later segments. Nevertheless, one can still inspect several Scatchard plots obtained in health and disease and examine the initial slope for an estimate of the affinity of the unoccupied receptor and the X intercept as an estimate of the maximum number of sites that may be determined if the receptor is involved in deviant physiology.

IV. Basic Receptor Models

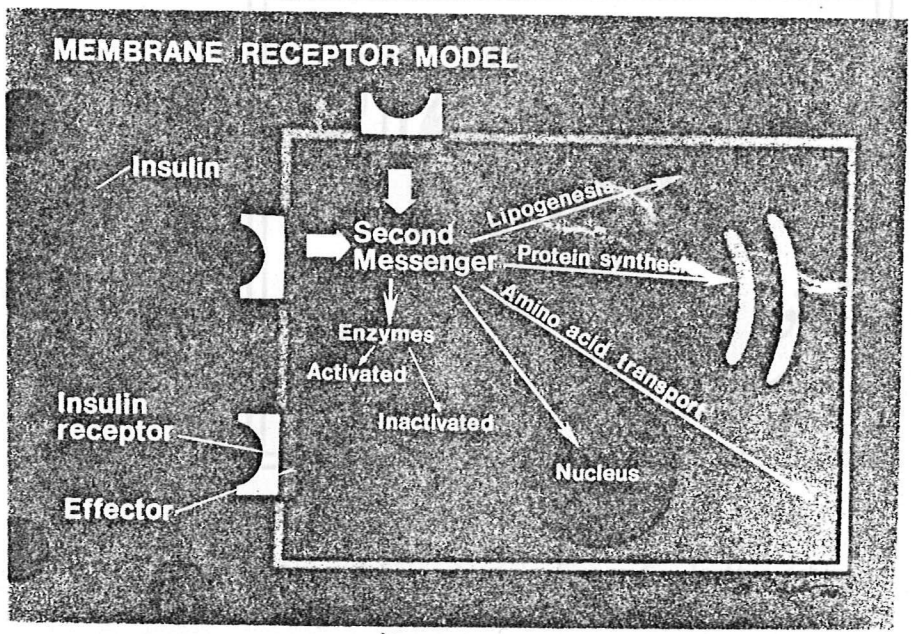
Three receptor models have been characterized to date as listed in Table 3.

Table 3 Receptor Models

I. Model one:	Receptor on the plasma membrane
II. Model two:	Receptor in the cytosol
III. Model Three:	Receptor in the nucleus

The first model is that of the receptor which resides in the plasma membrane of the cell, a location which characterizes the binding site for most polypeptide hormones and neurotransmitters. The cytosolic receptor model is characteristic of the binding sites of lipophyllic hormones such as the steroids. Recently a nuclear receptor has been described for thyroid hormone. Figure 5 is an artists schematic representation of the membrane receptor model.

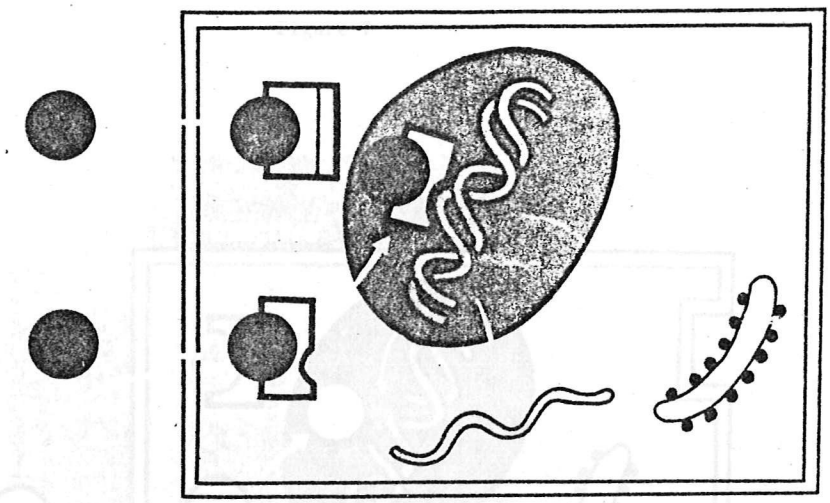
Figure 5



Each receptor in the plasma membrane is comprised of two subunits which often are chemically distinct. One subunit, the effector portion, links the binding event to the intracellular informational network, the second

messenger system. The other subunit is the ligand recognition region at which physical binding occurs. The ligand binds at the membrane activating the effector molecule which signals the cell through the second messenger to alter cellular function.

Figure 6



The cytosolic receptor model is shown in Figure 6. In this schematic one sees that the receptor molecule resides in the cytosol. In the Monad-Wyman-Changeux model, this receptor exists in an active and inactive form, the concentrations of which are in equilibrium in the basal condition. The agonist ligand moves easily through the cellular membrane because of the lipophyllic nature of the molecule and binds to the active

receptor species. The binding event shifts the equilibrium to favor this active variety. The hormone-receptor complex translocates to the nucleus where it binds to the genome to deliver its message, such as the initiation of messenger RNA synthesis culminating in new protein synthesis. An antagonist will bind preferentially to the inactive species of the receptor molecule shifting the equilibrium in that direction. The inactive receptor-hormone complex has a low affinity for critical nuclear sites and is unable to bring about induction at the genome level.

Figure 7

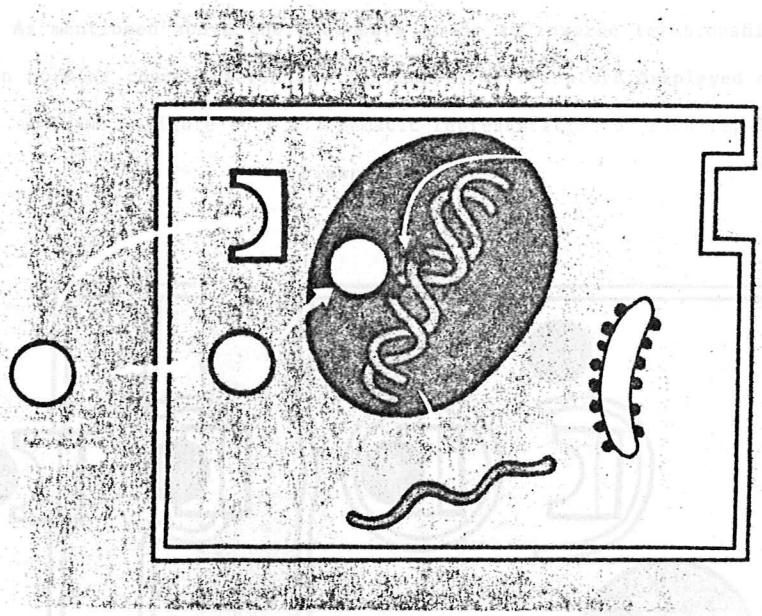


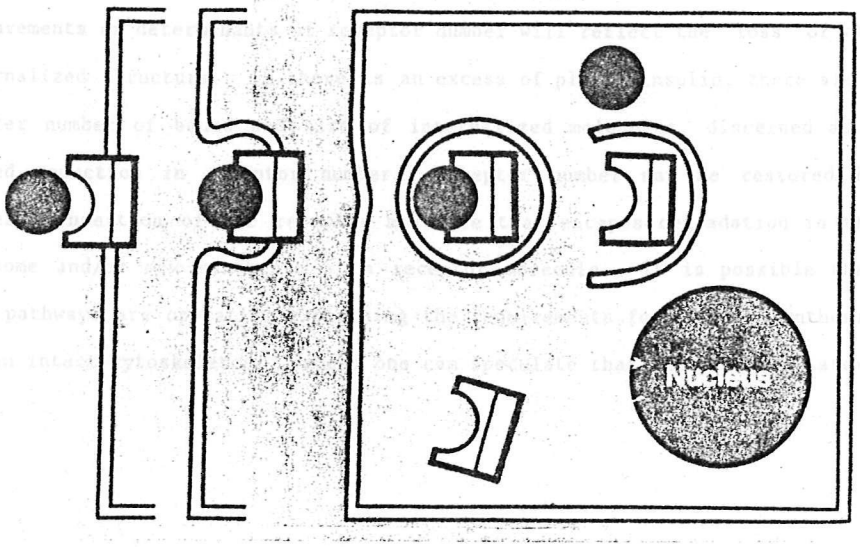
Figure 7 shows the interesting variation which is felt to represent the thyroid receptor system, a nuclear receptor model. Here one can see that thyroid hormone directly binds to spacer DNA passing its information to the cell. Depicted in this drawing is an interesting interaction between thyroid hormone and catechol receptors to which I will return later in this review. Interestingly an inactive cystolic binding site for thyroid hormone exists which can temper the effects of large concentrations of the hormone by binding prior to the movement of the hormone into the nucleus.

V. Regulation of hormone receptor number

A. Down regulation

As mentioned above there appears to be an inverse relationship between hormone concentration and the number of receptors displayed on the cell membrane. Figure 8 is a schematic representation of such regulation.

Figure 8



In the basal or normal physiologic state there is a given concentration of ligand and a finite number of receptors. If the ligand concentration is markedly increased, a cell exhibits fewer receptor sites. Some have felt this process of down-regulation initially may be a protective homeostatic mechanism in the face of hormonal excess. On the other hand if one can link ligand to receptor binding to hormonal function, then one may see how down regulation may play a role in the pathogenesis of certain hormone resistant states as will be discussed later. The mechanism of down regulation is as yet unclear but certain features are known. The process requires the binding event, cellular energy expenditure, an intact membrane cytoskeleton, and the presence of protein synthesis. One interesting hypothesis for the down regulation exhibited by the insulin receptor system is shown in Figure 8. The number of receptors displayed by a cell is an equilibrium between degraded and synthesized structures. When insulin binds to its receptors it is internalized through a process of endocytosis into a lysosome in which both the hormone and the receptor may be degraded. During the internalization process, that bound hormone cannot be displaced by another insulin molecule and that engaged receptor molecule is unavailable for a subsequent binding event. Binding measurements or determinants of receptor number will reflect the "loss" of the internalized structure. If there is an excess of plasma insulin, there are a greater number of bound and also of internalized molecules, discerned as a marked reduction in receptor number. Receptor number may be restored by either reinsertion of the receptor molecule that escapes degradation in the lysosome and/or new synthesis of a receptor molecule. It is possible that both pathways are operative explaining the requirements for protein synthesis and an intact cytoskeleton. Indeed one can speculate that receptor regulatory

signals may be received by the nucleus through a process of release of intact receptor-hormone complexes from the lysosome.

B. Up Regulation

For many systems the inverse relationship between ligand and receptor can be demonstrated in the up as well as down regulatory mode. Up regulation is extant when circulating concentrations of ligand fall and receptor number rises, perhaps as homeostatic protection against loss of necessary hormonal action to sustain life or health.

C. Heterotropic Regulation

The number of receptors for a given hormone may be altered by a second hormone not involved with the binding event to the site in question. Such heterotropic regulation has been briefly touched on previously in this review in the example of the thyroid hormone increasing the synthesis of catecholamine receptors potentially increasing cellular response to an unchanged concentration of circulating catechol.

D. The beta adrenergic receptor system: The classic model of receptor regulation explaining observed physiology

Lefkowitz and his colleagues, in an elegant series of studies, have shown that an understanding of the normal regulation of receptor number may explain many of the clinical features and observations that characterize the catechol system and which have been known to internists for many years. For example the repeated administration of a given agonist is known to result in decreasing effect, a phenomenon called tachyphylaxis. Clinically one can invoke tachyphylaxis to explain the decreasing

effectiveness of excessive use of bronchodilators or nasal sprays. Tachyphylaxis is now understood as an example of down regulation of the catechol receptor number on the target tissue. Drugs such as propranolol are therapeutic because they block the beta adrenergic receptor preventing the passage of catechol signals to the cell. A receptor occupied by this antagonist is not internalized and receptor number does not decline. Indeed the cell perceives the blocked state as one with reduced transmitter leading to the measurement of increased or new receptors. The sudden discontinuance of the propranolol can then lead to a hypercatechol response without a change in catechol levels, culminating in increase work of the heart and angina. Thus up regulation may explain the clinical rebound noted after the sudden cessation of propranolol. Lastly it is well known that many symptoms of hyperthyroidism are driven by catechols, yet catechol levels themselves are not much altered in that clinical disorder. This observation may be explained by the stimulation of the synthesis of catechol receptors by the excess thyroid hormone, an example of heterotropic receptor regulation.

VI. Receptors and Clinical Disease

Table 4 provides a working outline with which one can discuss the relationship of receptors to human disease.

Table 4

1. pre-receptor defects
2. receptor defects
3. post-receptor defects

Using the the receptor as the focus one can describe disorders which involve events that regulate ligand presentation to receptors which occur at a locus before ligand gets to the receptor, pre-receptor defects; disorders in which ligand-receptor interactions are normal but the cell fails to preceive that a binding event has occurred, post-receptor defects; or defects in the receptor itself.

It is of course highly artificial to examine endocrine disease from the point of view of the receptor alone. On the other hand such an artificial construct is useful for understanding and discussion. In this construct many disorders involve alterations in the ligand itself, the end result of which is to diminish the concentration of the ligand capable of engaging its receptor and passing its information to the cell. The regulation of ligand production is the most obvious example of a pre-receptor defect with none, more, or less hormone or neurotransmitter synthesized or released leading to disease. Production of the ligand may be normal but reduced presentation to the binding site may be the basis of altered physiology. Increased plasma carrier protein, anti-ligand circulating antibodies, or disordered presynaptic uptake pathways are all examples of this mechanism. I will not dwell further on diseases which have their genesis at the pre-receptor locus but will concentrate on receptor and post receptor defects in this review. Table 5 gives the format for my discussion of diseases in which altered receptor physiology plays an important role.

Table 5

Receptors and disease:

A. Anti-receptor antibodies

acanthosis nigricans (type B)
myasthenia gravis
Graves disease

B. Altered receptor molecule

androgen insensitivity
acanthosis nigricans (Type A)
severe subacute fast
lipodystrophy

C. Reduced number of receptor molecules

adult onset diabetes mellitus (Type II)
obesity

D. Absent receptor molecules

familial hypercholesterolemia

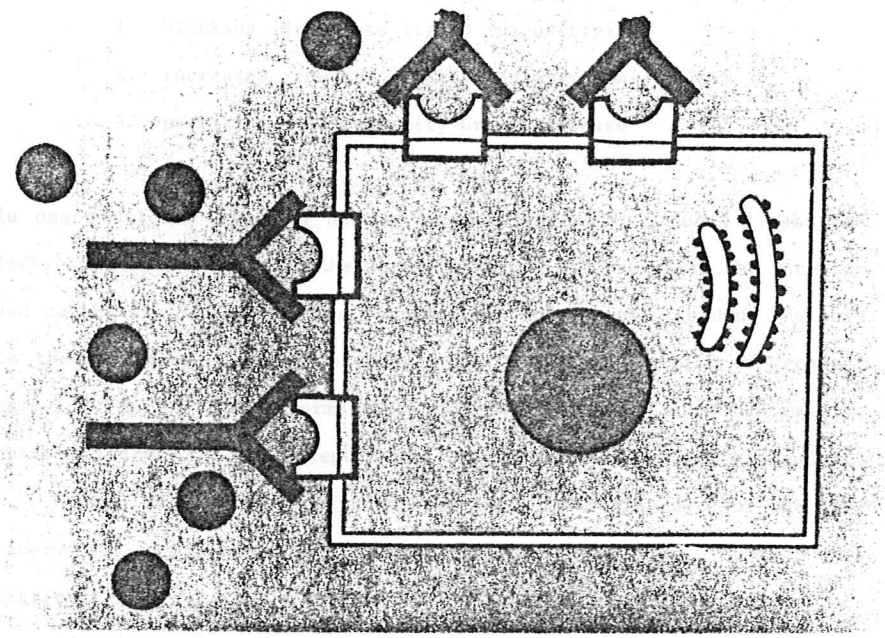
E. Post-receptor defects

leprechaunism
receptor positive, androgen resistance
obesity

A. Anti-receptor antibodies

The characterization of auto-antibodies directed against cell receptors is one of the most exciting discoveries in the receptor field in the last five years. The manner in which such antibodies may cause disfunction is shown in Figure 9.

Figure 9



The cell is equipped with its usual complement of receptors, each of which function normally. The recognition unit of the hormone receptor occupied by an antibody preventing engagement by the ligand can result in diminished ligand effect or a state of ligand resistance. Three seperate models of the anti-receptor antibody have been discerned.

Table 6

Three models of Anti Receptor Antibody Mediated Defects

1. blockade leading to ligand insensitivity
2. increased receptor degradation-myasthenia gravis
3. partial agonist activity-Graves disease

In one model the antibody blocks binding leading to reduced ligand effect. In another form of the defect, the antibody occupies the receptor and causes destruction of the receptor-antibody complex leading not only to the blockade of ligand binding but also to a reduction of receptor numbers. In the third formulation of this pathogenetic mechanism, the antibody binds to the receptor and acts itself as a partial agonist.

A form of acanthosis nigricans was the index disorder for the discovery and characterization of anti receptor antibodies. Acanthosis nigricans is a relatively common rash, described as a brown-black verrucous skin change found commonly in intertriginous zones. A unique form of this dermatosis, type B, is associated with severe insulin resistance defined by hyperinsulinism and hyperglycemia. Patients with the form of the dermatosis associated with the anti-receptor antibody tend to be middle aged females who have constitutional symptoms suggestive of an autoimmune syndrome. The erythrocyte sedimentation rate is elevated. Low titers of anti-nuclear antibodies are common, although anti-DNA is not found. Patients may have arthralgias, fever, and patchy alopecia. Flier and his colleagues have found a circulating antibody directed

specifically against the ligand binding unit of the insulin receptor, undoubtedly the cause of the insulin resistance and hyperglycemia of these patients.

Acanthosis nigricans Type B is a rare disease important for the discovery of anti-receptor antibodies and for its relationship to gastrointestinal malignancies. Excitingly Drachman and his associates at Johns Hopkins have recently shown the anti-receptor antibody mechanism to be responsible for the pathogenesis of the more common neurologic disorder, myasthenia gravis. Myasthenia is characterized by progressive weakness of the voluntary muscles during exercise or use. The disease fluctuates in severity, has a predilection for the cranial muscles with visual disturbance the most common early manifestation, and may lead to serious respiratory compromise. A diagnosis may be made by an electromyogram with the finding of progressive reduction in endplate potentials during external stimulus. Improvement after the administration of the short acting anticholinesterase edrophonium (tensilon; 2-10 mg IV) has been the classical clinical test for the disease.

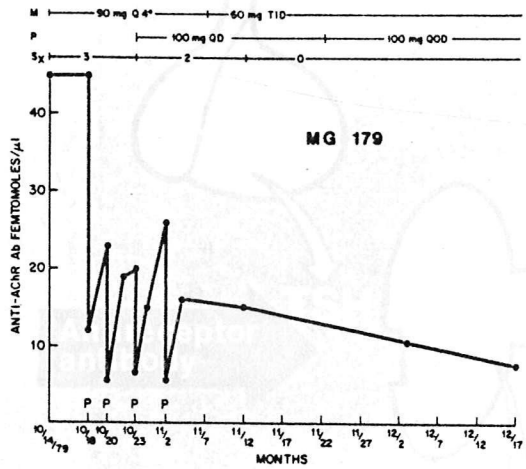
The autoimmune nature of myasthenia gravis has long been suspected. There is an association with certain neoplasms and especially with thymomas. Anti-nuclear, anti-thyroid, anti-muscle antibodies have been found. A decreased number of T cells, an increased number of B lymphocytes, and the auto reactivity of lymphocytes in the mixed lymphocyte culture have all been taken as evidence to support enhanced lymphocyte activity the result of abnormal T cell regulation. Deranged and diminished T cell suppressor activity could explain all the immunologic find-

ings. Building on an animal model of experimental allergic myasthenia gravis in which anti-acetylcholine antibodies raised specifically caused a myasthenia like syndrome, Drachman was able to define complement fixing IgG antibody in patients with myasthenia for which human acetylcholine receptor was the antigen. The antibody can be shown to disrupt neurotransmitter coupling of nerve to muscle in more ways than single blockade of the ligand binding site. The antibody molecule, being polyvalent, crosslinks several receptor molecules, effectively reducing receptor number. Moreover the presence of antibody on the receptor excelerates receptor degradation.

The antibody nature of myasthenia gravis explains several curious features of the disorder. For example short lived passive transfer in animal models is now understandable in terms of administration of the anti-receptor antibody in serum. Most importantly antibody directed pathogenesis permits a rational approach to the therapy of myasthenia. The classical treatment by acetylcholine esterase inhibitor drugs may be effective in less severe cases in that these agents prevent acetylcholine breakdown providing increased ligand for binding to the decreased number of receptor sites. Steroids as immunosuppressive drugs have been shown to be effective in 70-100% of patients in various recent series. If antibodies cause the disease then removal of plasma containing the offending globulin should be beneficial. Figure 10 provides data from a patient treated recently at Southwestern Medical School by Dr. Richard Tindall and his colleagues in which the clinical benefit of plasmaphoresis is readily apparent.

Figure 10

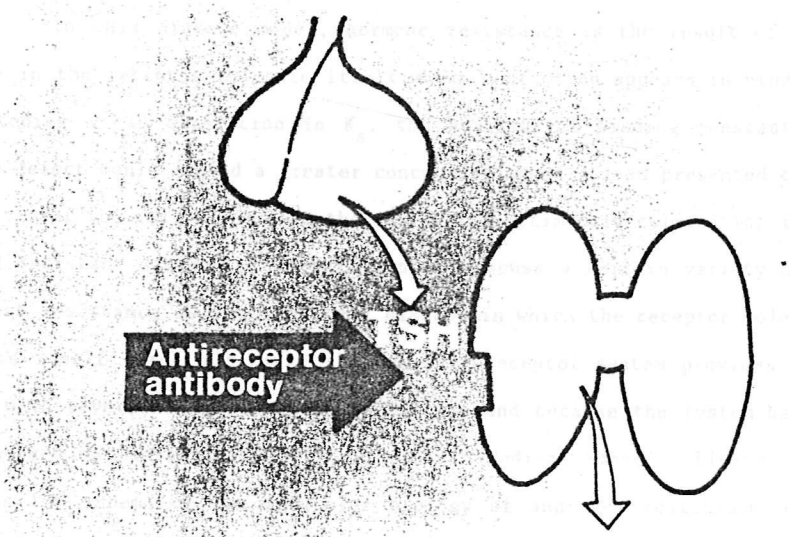
Anti-acetylcholine antibody titers during plasmaphoresis in a patient treated with prednisone (P) and mestinon (M). The number under SX indicates severity of symptoms.



The anti-receptor antibody found in the circulation of patients with the variety of hyperthyroidism described by Graves illustrates the third way in which anti-receptor antibodies cause disease. The binding of antibody to the receptor serving as antigen may not always be an inert event. Rather it is possible that the occupation of the recognition portion of a receptor molecule by the antibody may trigger the coupling reaction and initiate a cellular response equivalent to, or greater than, ligand binding itself. Figure 11 schematically depicts this mechanism for Graves disease as worked out by Hall and his associates.

Figure 11

The pathogenesis of Graves Disease - the role of anti-TSH receptor antibody



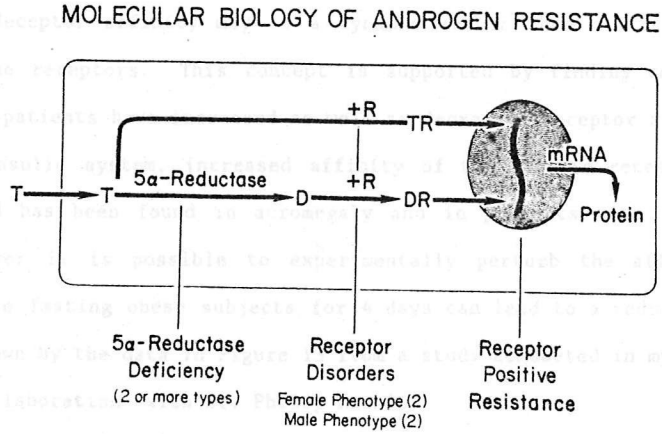
Thyroid hormone synthesis is controlled by the pituitary tropic hormone thyroid stimulating hormone, TSH. TSH binds to a membrane receptor on the thyroid gland, stimulates adenylate cyclase and thus enhances the thyroid cell synthetic activity. Among the series of unusual globulins synthesized by patients with Graves disease is an antibody which recognizes and binds to the TSH membrane bound receptor on the thyroid gland. This antibody itself is fully capable of stimulating adenylate cyclase, the second messenger coupling TSH binding to enhanced thyroid hormone synthesis. The usual negative feedback relationship between tropic

Although thyroid hormone may actually turn off pituitary elaboration of TSH, pseudo-TSH, the antibody, is under no such feedback control. The result is autonomous, TSH-independent, hyperfunction of the thyroid gland.

B. Altered receptor molecule

In this disease model, hormone resistance is the result of a change in the receptor molecule itself which most often appears in binding studies as an alteration in K_a, the equilibrium binding constant. Such a defect would demand a greater concentration of ligand presented to the cell for the cell to perform the same task, ultimately culminating in ligand resistant states. I have chosen to discuss a certain variety of androgen resistance as the prototype disease in which the receptor molecule is itself altered because the androgen receptor system provides a model with several types of receptor defects and because the system has been worked out so well here at Southwestern Medical School. Figure 12 depicts the schema of the molecular biology of androgen resistance as worked out by Griffin and Wilson.

Figure 12

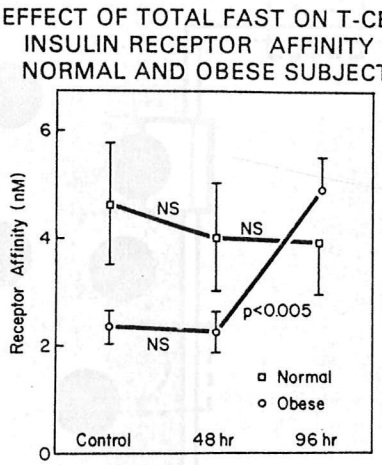


The androgen steroid easily moves into the cell where circulating testosterone is converted to the more active dihydrotestosterone by the enzyme 5α reductase. As for all steroid hormones, the receptor resides in the cytosol, binding the hormone and passing its information to the nucleus. Griffin and Wilson have found patients with androgen resistance presenting as testicular feminization who have peculiarly thermolabile androgen receptors. This alteration in the molecule may lead to in vivo receptor destruction accounting for the hormone resistance exhibited in this disease.

An unusual disease associated with insulin resistance shown in many cases to be related to a large decrease in receptor affinity is lipodystrophy. Patients so afflicted exhibit a generalized loss of adipose tissue, increased plasma insulin, hepatosplenomegaly and hypermetabolism. The reduced hypoglycemic response to insulin in these patients defines the state of hormone resistance. Oseid and colleagues as well as Rosenbloom et al, studied insulin receptor characteristics on several different tissues finding uniformly reduced ligand binding the consequence of diminished receptor affinity. The chemical nature of the receptor defect is not yet apparent.

Receptor affinity may be a dynamic rather than static quality of hormone receptors. This concept is supported by finding conditions in which patients have increased as well as decreased receptor affinity. In the insulin system, increased affinity of the insulin receptor for its ligand has been found in acromegaly and in patients with insulinomas. Moreover it is possible to experimentally perturb the affinity. For example fasting obese subjects for 4 days can lead to a reduced affinity as shown by the data in Figure 13 from a study conducted in my laboratory in collaboration with Dr. Philip Raskin.

Figure 13

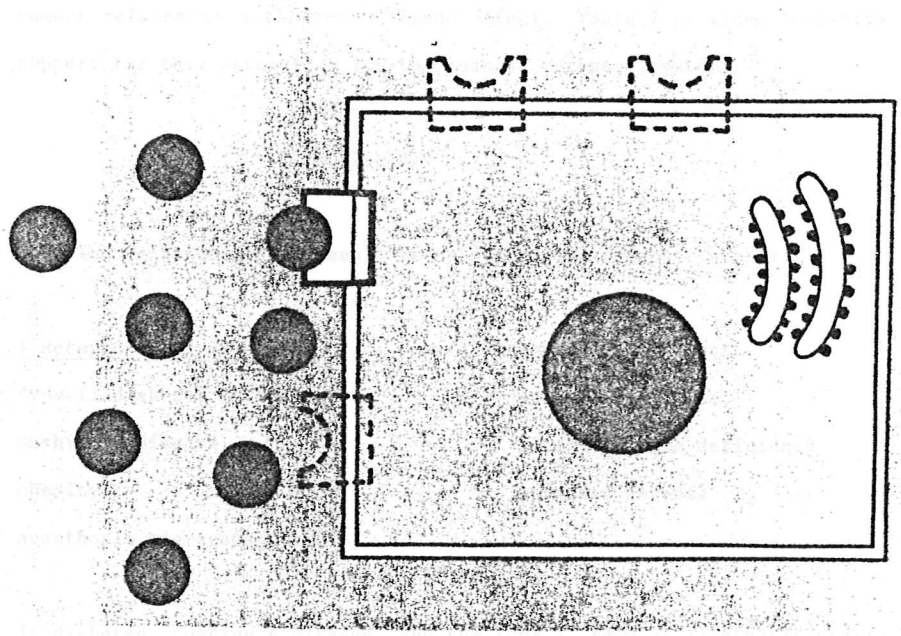


Similarly the simple administration of glucose loads may be a signal to increase affinity, teleologically to insure more efficient disposition of a subsequent load.

C. Reduced Number of Receptor Molecules

Figure 14 is a cartoon representation of a disorder characterized primarily by a reduction in the number of receptors leading to hormone resistance.

Figure 14



In this model the amount of ligand is normal or even increased. The cellular machinery coupling binding to cellular response can be shown to be normal in pure forms of this defect, since there is a normal cellular response to exogenously provided ligand in an adequate concentration. Radioligand binding studies reveal diminished binding, the consequence of reduced total available receptor sites. There is an expanding list of diseases associated with reduced receptor members. I will return to the now familiar insulin receptor system for two prototypic diseases to illustrate several points that are operative in this mechanism of altered physiology, Type II diabetes mellitus and the more complex syndrome, obesity.

Any discussion that seeks to relate deviation in receptor number to pathogenesis must begin with the assumption that a change in receptor number relates to a change in ligand effect. Table 7 provides tentative support for this assumption for the insulin receptor system.

Table 7

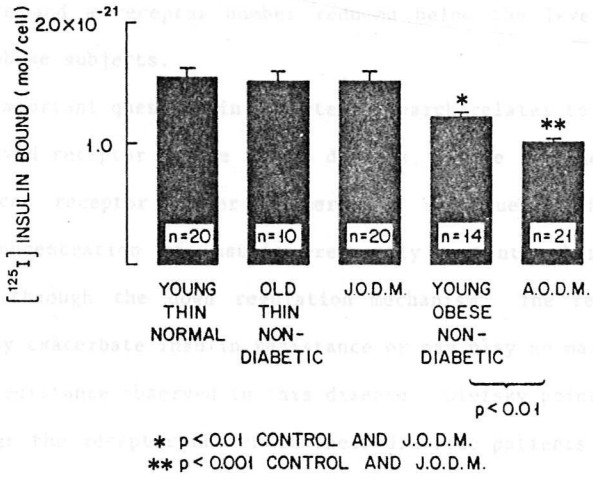
The relationship between hormone effect and receptor number.

↓ Receptor #, ↓ Effect	↑ Receptor #, ↑ Effect
type II diabetes mellitus	Addison's disease
Cushing's disease	growth hormone deficiency
obesity	anorexia nervosa
acanthosis nigricans (type A)	

In diabetes, Cushing's disease, and the Type A form of acanthosis nigricans, one can measure cellular insulin resistance in the face of decreased number of cell insulin receptors. Conversely in patients with Addison's disease, dwarfism with growth hormone deficiency, or anorexia nervosa, one can measure heightened insulin sensitivity associated with an increased receptor number. Such clinical observations permit an in depth analysis of the relationship of receptor number and disease.

Figure 15 is a survey of the insulin binding capacity on cells from a cross section of the Parkland diabetes clinic patients with matched controls.

Figure 15 - Insulin receptor binding in patients with various carbohydrate disorders



Note that normal aging does not effect insulin binding in thin, nondiabetic individuals. Insulin binding capacity is also perfectly normal in patients in our clinic with Type I (juvenile onset) diabetes mellitus.

Young obese, non diabetic control subjects exhibit reduced binding entirely the result of fewer insulin receptors. This review will return to obesity shortly. Type II diabetics, weight matched to the obese group, displayed the least insulin binding, consequent to a fall in insulin receptor number of almost 80%. Our data support the pre-receptor nature of Type I diabetes. There is no insulin resistance and no receptor defects. Conversely in Type II diabetes, there is cellular insulin resistance and a receptor number reduced below the level exhibited by matched obese subjects.

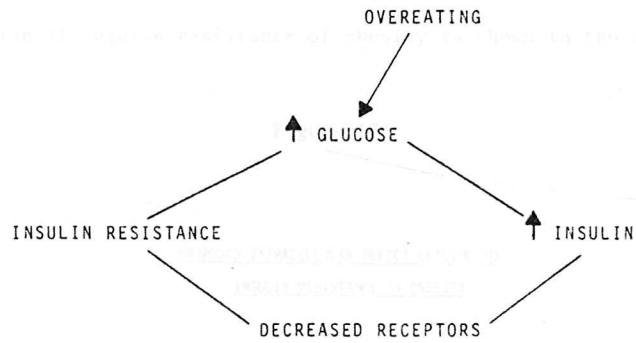
An important question in diabetes research relates to the primacy of the observed receptor change in the disease. There is some evidence that the reduced receptor number is merely a consequence of the elevated plasma concentration of insulin frequently encountered in the Type II diabetic through the down regulation mechanism. The reduced receptor number may exacerbate insulin resistance or may play no major role in the insulin resistance observed in this disease. Olefsky points out that one can change the receptor number in these diabetic patients by such interventions as composition of diet, without ameliorating the diabetic state. On the other hand, there is some evidence that the reduced number of insulin receptors in the Type II diabetic in the unperturbed state may be an intrinsic, even inherited, characteristic of these patients.

The argument whether insulin receptor determination is causal of, an important contributer to, or an epiphenomenon of obesity is also as yet unsettled. The Roth group at the NIH have argued that the reduction in receptor number is secondary to a central hyperphagic drive but itself is casual of the insulin resistance observed in the obese state. Figure 16 from one of their reviews portrays this hypothesis.

Figure 16

OBESITY: RECEPTOR DEFECT CAUSAL

ROTH ET AL.

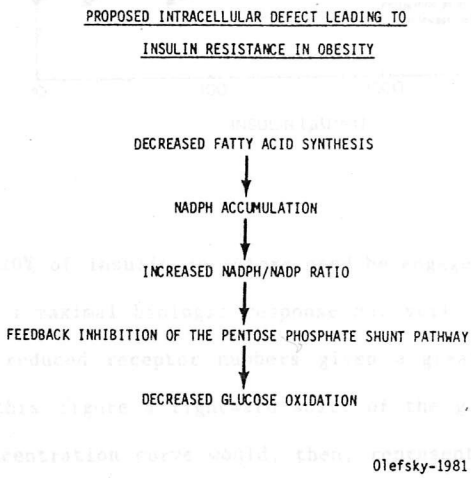


In obesity it is felt that there is a derangement in the hyperthalamic control of eating. Hyperphagia results in elevation in plasma glucose which stimulates the beta cell of the pancreas to synthesize and secrete excess insulin to dispose of the increased load. At the cellular level the hyperinsulinism down regulates the number of insulin receptors which leads to relative insulin resistance. The chronic turning of this pathologic circle culminates in a pathological state characterized by hyperinsulinism, hyperglycemia, and tissue insulin resistance.

New data permits a challenge to the notion that the decrease in insulin receptors is causal of the insulin resistance found in obesity. Using an animal model of spontaneous obesity associated with a defect in carbohydrate metabolism similar to that observed in man, Olefsky showed that cells from obese animals exhibited normal rates of insulin directed glucose transport while having a marked attenuation in insulin-supported

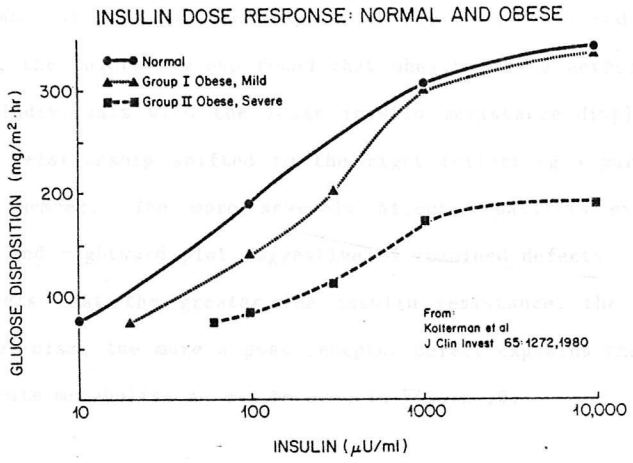
glucose oxidation. Olefsky argues that the primary metabolic defect in obesity may have an intracellular locus. In my construct, obesity is viewed as a post receptor defect. Persuasive evidence is available to implicate diminished fatty acid synthesis as the culprit in the production of insulin resistance of obesity as shown in the schema in Figure 17:

Figure 17



In order to determine the relative contributions of receptor and post receptor defects to the insulin sensitivity in obesity it is necessary to apply a dose response relationship of insulin and glucose disposition in human obesity. Figure 18 from Kolterman and Olefsky describe just such a relationship.

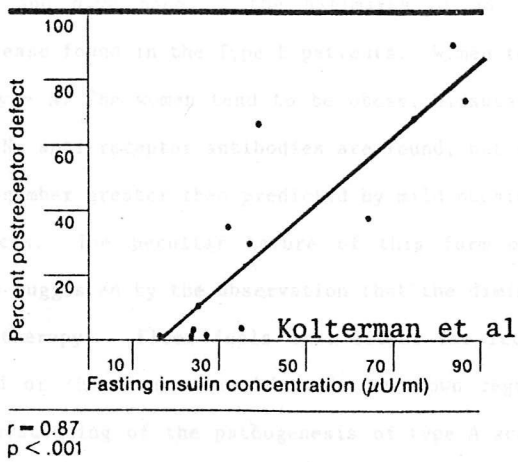
Figure 18



Since only 20% of insulin receptors need be engaged for maximal biologic responsive, a maximal biologic response may still be forthcoming in the setting of reduced receptor numbers given a greater insulin concentration. In this figure a rightward shift of the glucose disposal to log insulin concentration curve would, then, represent a patient with cells exhibiting receptor reduction. Conversely, if post receptor defects exist in the absence of altered receptor kinetics, there would be a reduction in the glucose disposal rate exhibited at maximally effective insulin levels. The dose response curve in this setting would not be shifted to the right but the maximal achievable rate of glucose disposition would be far below that of the normal, a shift downward. Finally patients with combined defects will display both a rightward shift of the

curve indicative of a reduction in receptor number and a downward shift indicative of submaximal disposal rate at maximally effective hormone doses. When in vivo glucose disposition rates were measured in 13 obese subjects, the Colorado group found that obesity was a heterogenous disorder. Individuals with the least insulin resistance displayed a dose response relationship shifted to the right reflecting a pure defect in receptor member. The more severely affected patients exhibited the downward and rightward plot suggestive of combined defects. The Olefsky group feels that the greater the insulin resistance, the greater the hyperinsulinism, the more a post receptor defect explains the disordered carbohydrate metabolism as can be seen in Figure 19.

Figure 19



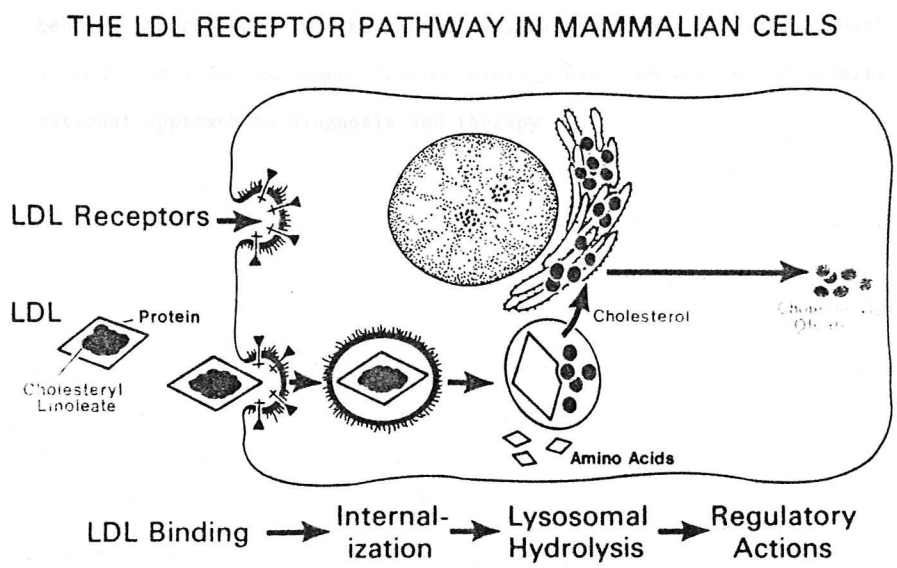
Although the issue is still under study, one concludes at this time that obese patients with mildly elevated insulin levels have mild hormone resistance secondary, in great measure, to a reduction in insulin receptors, while the severely resistant patients with hyperglycemia and severe hyperinsulinism have a combined defect with post receptor intracellular defects predominant.

Before leaving a discussion of the prototypic diseases associated with reduced receptor numbers, I would like briefly to discuss a third example of altered carbohydrate metabolism associated with a reduction in receptor number of a peculiar type. This disease represents a second type of glucose intolerance associated with the characteristic blackish skin rash acanthosis nigricans called type A. These patients stand in stark contrast to the patients afflicted with the Type B form of the dermatosis described previously in the discussion of anti receptor antibody mediated disorders. The patients with Type A are young rather than middle aged and have none of the hallmarks of an active immunologic directed disease found in the Type B patients. Women tend to predominate as in the Type A. The women tend to be obese, hirsute, amenorrheic, and infertile. No anti-receptor antibodies are found, but rather a reduction in receptor number greater than predicted by mild obesity is displayed by these subjects. The peculiar nature of this form of receptor number reduction is suggested by the observation that the diminution is reversed by insulin therapy. Flier feels that either the receptor molecule is also altered or there is a breakdown in the down regulatory mechanism. Further understanding of the pathogenesis of type A acanthosis nigricans awaits chemical determination of the receptor molecule by solubilization studies.

VI. Absent Receptor Molecules

It is intuitively obvious that the complete absence of receptor binding molecules negates ligand directed cellular activity. There can be no better example of a human disease the pathogenesis of which is directly the consequence of absent binding molecules than that of familial hypercholesterolemia. Goldstein and Brown have shown that cellular metabolism of cholesterol requires the binding of low density lipoproteins (LDL) carrying cholestorol to a receptor on the cell membrane as shown in Figure 20 from their work.

Figure 20



The LDL binding at specific sites in coated pits on the cell membrane leads to internalization, lysosomal hydrolysis, and then appropriate regulatory activity. The absence of the LDL receptor as shown by those two investigators in the homozygous form of the disorder is causal of the severe hypercholesterolemia found which is ultimately related to early atherogenesis and death.

VII. Summary

I have constructed a schema for discussing receptor pathophysiology using prototypic clinical syndromes as examples of the utility of the the construct. I have reviewed basic receptor physiology in order to create a familiarity of, and appreciation for, new approaches to understanding bedside observations and the pathogenesis of disease. Such a mechanistic approach to selected human disease expands basic knowledge and permits a rational approach to diagnosis and therapy.

REFERENCES

The references listed below were chosen because they provided the most thorough recent review of a topic discussed in these rounds or contained material directly cited.

1. Bar R.S. and Roth J. Insulin receptor status in disease states of man. Arc Int Med 137:474, 1977.
2. Bar R.S., Harrison, L.C. and Muggeo, M. et al. Regulation of insulin receptors in normal and abnormal physiology in humans, In Advances In Internal Medicine Vol. 24, (ed) G.H. Stollerman (Year book Medical publisher, Chicago, 1979) p. 23.
3. Beck-Nielsen, H. The pathogenetic role of an insulin receptor role in the diabetes of obese. Diabetes, 27:1175, 1978.
4. Böynaems, J.M. and Dumont, J.E. Quantitative analysis of the binding of ligands to their receptors. J Cycl Nuc Res, 1:123, 1975.
5. Brown, M. and Goldstein, J. Familial hypercholesterolemia: Model for genetic receptor disease. The Harvey Lecture Series, 73 (New York City, 1979).
6. Czech M.P. Molecular basis of insulin action. Ann Rev Biochem, 46:359, 1977.
7. Czech, M.P. Cellular basis of insulin insensitivity in large rat adipocytes. J Clin Invest, 55:1523, 1976.
8. Cuatrecasas, P. Membrane receptors. Ann Rev Biochem 43:169, 1974.
9. DeMeyts, P., Cooperative properties of hormones receptors in some membranes. J S Molecular Structure 4:241, 1976.

10. Demeyts, P. Roth, J. Cooperativity in ligand binding, a new graphic analysis. Biochem Biophys Res Comm, 66:1118, 1975.

11. Drachman, D.B. Myasthenia gravis. N Eng J Med, 298:136, 1978 and N Eng J Med, 298:186, 1978.

12. Flier, J.S., Kahn, C.R., Jarrett D.B. et al. Characterization of antibodies to the insulin receptor. A cause of insulin resistant diabetes in man. J Clin Invest, 58:1442, 1976.

13. Ginsberg, B.H. The insulin receptor: Properties in regulation. In Biochemical Actions of the Hormones, Vol. 4, (ed) Litwack, P. (NY Academic Press, 1977), p. 313.

14. Glaubiger, G. and Lefkowitz, R.J. Elevated beta adrenergic receptor after propranolol treatment. Biochem Biophys Res Comm 78:720, 1977.

15. Griffin, J.E. and Wilson, J.D. The syndromes of androgen resistence. N Eng J. Med, 302:198, 1980.

16. Helderman, J.H., and Raskin P. The T-lymphocyte insulin receptor in diabetes and obesity: An intrinsic binding defect. Diabetes, 29:551-557, 1980.

17. Helderman, J.H. and Strom, T.B. The emergence of insulin receptors upon alloimmune cells in the rat. J Clin Invest 59:338-344, 1977.

18. Hollenberg, M.D. Receptor models and the action of neurotransmitters in hormones. In Neurotransmitter Receptor Binding, (ed) H.I. Yamamura, et al. (Raven Press, NY, 1978) p. 13.

19. Hollenberg, M.D. and Cuatrecasas, P. Insulin: interaction with membrane receptors in the relationship to purine nucleotides and cell growth. Fed Proc 34:1556, 1975.

20. Hollenberg, M.D. and Cuatreacasas, P. Membrane receptors and hormone action: recent developments. Proc Neuro Psycho Pharm 2:287, 1978.

21. Jacobs, S., Chang, K.J. and Cuatrecasas, P. Estimation of hormone receptor affinity by competitive displacement of labeled ligand: Effect of concentration of receptor and of labeled ligand. Biochem Biophys Res Comm 66:687, 1975.

22. Kahn, C.R. Membrane receptors for hormones and neurotransmitters. J Cell Biol 70:261, 1976.

23. Kahn, C.R., Flier, J.S., Bar, R.S., et al. The syndrome of insulin resistance and acanthosis nigricans. Insulin receptor disorders in man. N Eng J Med 294:739, 1976.

24. Kahn, C.R., Megyesi, K., Bar, R.S., et al. Receptors for peptide hormones. New incites into the pathophysiology of disease states in man. Ann Int Med 85:205, 1977.

25. King, A.C., Cuatracasas, P. Peptide hormone induced receptor mobility, agregation, and internalization. N Eng J Med 305:77, 1981.

26. Krupp, M., and Lane M.D. On the mechanism of ligand induced down regulation of insulin receptor in the liver cell. J Bio Chem 256:1689, 1981.

27. Lefkowitz, R.J. Mukhergee, C. Lombard, L.E., et al. Regulation of adenylate cyclase coupled beta adrenergic receptor. Recent Prog Horm Res 32:597, 1976.

28. Lefkowitz, R.J. Isolated hormones receptors. Physiologic and clinical implications. N Eng J Med 288:1061, 1973.

29. Lockwood, D.H. Insulin resistance in obesity. Hospital Practice, p. 79, December, 1976.

30. Marshall, S.M. and Olefsky, J.M. Effects of lysomotropic agents on insulin interaction with adipocytes. J Biol Chem 54:10153, 1979.

31. Olefsky, J.M. Effects of fasting on insulin binding, gluclose transport oxidation and isolated rat adipocytes. J Clin Invest 58:1450, 1976.

32. Olefsky, J.M. The insulin receptor: Its role in insulin resistance in obesity and diabetes. Diabetes 25:1154, 1976.

33. Olefsky, J.M. The Lilly lecture. 1980: Insulin resistance and insulin action: an in vitro and in vivo perspective. Diabetes 30:148, 1981.

34. Olefsky, J.M., and Kolterman, O.G. Mechanisms of insulin resistance in obesity and non-insulin, dependent diabetes. N Eng J Med 70:151, 1981.

35. Olefsky, J.M. The insulin receptor: Physiology in pathophysiology In Current Concepts (The Upjohn Company, Kalamazoo, 1980).

36. Oseid, S., Beck-Neilson, H., Pedersen, O. Decreased binding of insulin to its receptors in patients with congenital generalized lipodystrophy. N Engl J Med 296:245, 1977.

37. Pollet, R.J., Standaert, M.L. and Haase, B.R. Insulin binding to the human lymphocyte binding receptor: Evaluation of the negative cooperativity model. J Biol Chem 252:5828, 1977.

38. Pollet, R.J. and Levey, G.S. Principles of membrane receptor physiology and their application to clinical medicine. Ann Int Med 92: 663, 1980.

39. Rabinowitz, D. Some endocrine and metabolic aspects of obesity. Ann Rev Med, 21:241, 1970.

40. Reaven, G.M., Olefsky, J.M. The role of insulin resistance in pathogenesis of hypoglycemia. In Advances In Modern Nutrition: Vol. 2 Diabetes, Obesity and Vascular Diseases Part I, (eds) Katzen H.M. Katzen and Mahlar, R.J. (N.Y. Holsted Press: 1978) p. 226.

41. Rodbard, D. Mathematics of hormone-receptor interaction. I. Basic principles. Adv Exp Med and Biol 36:289, 1973.

42. Roth, J., Kahn, C.R., Lesmiak, S., et al. Receptors for insulin, MSILA-s, and growth hormone: Applications to disease states in man. Recent Prog Horm Res 31:95, 1976.

43. Scatchard, G. The attractions of proteins for small molecules and ions. Ann NY Acad. Sci 51:660, 1949.

44. Suzuki, K. and Kono, T. Internalization and degradation of fat cell bound insulin. J Biol Chem 254:9786, 1979.

45. Taylor, S.I. Binding of hormones to receptors. An alternative explanation of non-linear Scatchard plot. Biochemistry 14:2357, 1975.

46. Tell, G.P., Haour, F., and Saez, J.N. Hormonal regulation of membrane receptors in cell responsiveness: A review. Metabolism 27:1566, 1978.

47. Verhoeven G.F.M. and Wilson, J.D. The syndromes of primary hormone resistance. Metabolism 28:253, 1979.

48. Williams, L.T., Lefkowitz, R.J. Receptor binding studies in adrenergic pharmacology (NY, Raven Press: 1978).

49. Williams, L.T., Lefkowitz, R.J. and Hathaway, D.R., et al. Thyroid hormone regulation of beta adrenergic receptor number. J Biol Chem 252:2787, 1977.