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## MEMBRANE RECEPTORS FOR HORMONES AND NEUROTRANSMITTERS

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### I. INTRODUCTION

Over the past 20 yr, evidence has come from a variety of sources to suggest that the primary event in the action of peptide hormones<sup>1</sup> and some neurotransmitters is binding to a specific site on the plasma membrane of the cell known as a receptor site. Most early studies attempted to define these hormone receptors by studying the steps of hormone action in reverse order, i.e. by investigation of effects of various factors on hormonally induced biological responsiveness in intact animals or intact tissues. More recently, with development of methods for preparation of high specific activity, biologically active labeled hormones, and hormonally responsive cells or cell membranes, direct study of hormone-receptor interactions has become possible. Such studies have now been employed for a wide variety of tissues and a large number of peptide hormones and neurotransmitters (for a list see reference 73). These studies have resulted in a significant increase in our understanding of the chemical nature of receptors and how they interact with their hormonal ligands. Many aspects of the hormone-receptor interaction have been discussed in previous reviews (11, 19, 22, 73, 106, 123, 130). The purpose of this review is to bring together some concepts about the hor-

<sup>1</sup> The references and figures accompanying this article are not meant to be comprehensive and have been selected only to illustrate certain points. More detailed reference lists are found accompanying those reviews.

mone receptor as a component of the cell membrane, the chemical nature of hormone receptors, and cellular regulation of hormone receptors as they relate to the biology of the cell. Only the receptors for peptide hormones and for the cholinergic and the  $\beta$ -adrenergic neurotransmitters will be considered. Receptors for viruses, immunoglobulins, and drugs demonstrate many features similar to those of the hormone receptors; however, a review of these is outside the scope of this article.

### II. METHODOLOGICAL CONSIDERATIONS

The basic methodology employed for most direct studies of the hormone-receptor interaction is that used in other competitive protein binding assays (73). An isotopically labeled hormone (hormone\*) or hormone competitive antagonist is incubated with a suitable receptor preparation. After some period of time the hormone\*-receptor complex is separated from the free hormone\* by centrifugation, filtration, or precipitation, and the receptor bound radioactivity is determined. The specificity and saturability of this reaction are demonstrated by performing the incubation with various amounts of unlabeled hormone or related compounds. A few studies have also been conducted with photoaffinity probes (46) or with fluorescent (13) or ferritin-labeled ligands (71, 110), but these latter techniques have not been widely applied. Methodologic problems related to choos-

ing the appropriate labeled hormone, receptor preparation, and technique of separation have been reviewed elsewhere in detail (73); however, several points regarding the hormone and receptor preparations deserve comment here.

In general, direct studies of the peptide hormone-receptor interaction have employed labeled agonists as the ligand in studying the binding reaction. In studies of the receptors for the neurotransmitters, labeled competitive antagonists have proved more useful. In the case of the  $\beta$ -adrenergic receptor, the labeled antagonist provides the analogue and stereochemical specificity not observed in studies with labeled agonists (2, 14, 103). With the cholinergic receptor, antagonists such as snake venom  $\alpha$ -toxins have proved useful since they bind in an almost irreversible fashion to the receptor (19, 99, 106, 119), although they may occupy twice as many sites as acetylcholine itself (119). In both cases, the reasons for the discrepancies are not clear, but obviously reflect the fact that the agonist and antagonist are not binding to identical populations of sites.

Of available radioisotopes,  $^{125}\text{I}$  is the most useful for labeling peptide hormones. Tracers prepared with one atom of  $^{125}\text{I}$  per molecule have a sp. act. of about 2,200 Ci/mmol (73). In general, it appears that many of the monoiodohormones, including even molecules as small as  $\beta$ -adrenergic antagonists, retain all or almost all of their biological activity (14, 73). Examples of both decreased and increased biological activity of labeled hormones may be found, however (73).

Tissue preparations used as a source of receptors have included intact cells, particulate fractions of cells, and "solubilized" fractions of cells. Each offers certain advantages and disadvantages. Isolated intact cell preparations have been obtained from blood, tissue culture, or by enzymatic or mechanical disruption of tissues. These offer a major potential advantage in that they are usually metabolically active, making it possible to correlate hormone binding and biological effect (15, 41, 51, 82, 129, 153). On the other hand, a number of undesirable side reactions such as hormone degradation may be higher with intact cells (45). Furthermore, the types of enzymatic digestion (often crude collagenase, hyaluronidase, or trypsin) used to isolate cells can profoundly affect the concentration or affinity of a number of different hormone receptors (73, 82, 153). It is important to keep in mind the heterogeneity of cell types which compose most tissues (which may or may

not be morphologically distinguishable) and that the receptor under study may be restricted to a minority of the elements of the preparation (134, 154). Affinity-labeling and autoradiography are particularly useful in identifying the receptor-containing cells in mixed populations (134, 154). Cells in tissue culture offer the greatest ease in isolation; even here, however, the process of adapting to culture, stage in cell cycle, and possible transformation may affect the receptor population (see section VII).

Particulate cell fractions often possess hormonally responsive enzymes and offer an additional advantage with regard to stability during storage. In general, there is a distinct value in using more purified and well-characterized membrane preparations, since crude particulate fractions contain internal organelles which may possess specific, as well as nonspecific, hormone-binding sites, and hormone-degrading sites (*vide infra*) (105). It is important to emphasize, however, that every plasma membrane isolation procedure is a selection process in which only 10–50% of the total plasma membrane is obtained. Thus, a given membrane preparation may not be representative of the total plasma membrane. Furthermore, if the membrane preparation obtained is from a tissue homogenate, the various cell types present may contribute preferentially to the final membrane preparation. For this reason, every membrane preparation must be assessed for yield, purity, and contamination with other organelles, especially when attempting quantitative comparisons in various physiological and pathological states (105).

Solubilized fractions of cells and cell membranes have proven most useful for attempts at purification and chemical characterization of the hormone receptor. Neutral and anionic detergents, anionic bile salts, phospholipid emulsions, lithium diiodosalicylate, and even limited proteolytic digestion have been used (21, 33, 52, 81, 106, 119, 136, 146). Solubilized insulin receptors have also been obtained from cultured human lymphocytes without detergents (48). While many hormone receptors appear stable to solubilization, changes in both affinity (81, 136) and number (22) have been reported, presumably due to alterations in conformation or uncovering of "masked receptors" by disruption of membrane phospholipids. In addition, the solubilized receptor suffers all of the problems of heterogeneity and contamination that the original insoluble receptor possessed. Thus, it is necessary to establish the char-

acteristics of the hormone binding site in the soluble preparation, so that nonspecific binding sites and degrading enzymes are distinguished from the true receptors. Finally, it should be recognized that it is erroneous to assume that the soluble receptor has been reduced to its fundamental state; suspensions of small membrane vesicles or fragments or detergent micelles which contain several proteins may fulfill the criteria for solubilization and may be mistaken for an "isolated," soluble receptor in the hormone-binding studies.

### III. IDENTIFICATION OF THE RECEPTOR

There is general agreement that the term "receptor" refers to a molecule (or molecular complex) which is capable of recognizing and selectively interacting with the hormone or neurotransmitter, and which, after binding it, is capable of generating some signal that initiates the chain of events leading to the biological response. Since receptors for peptide hormones and neurotransmitters have not yet been identified as purified chemical entities, however, some confusion has resulted over what functional characteristics should be used to define them. This problem in identification has led to a series of semantic arguments and even several papers on the "molecular linguistics" of hormone action (11, 61). Some investigators have suggested that the term receptor be reserved for situations in which both components of this bifunctional element have been demonstrated, i.e. hormone binding and biological response, and that a hormone-binding site detected in the absence of a defined biological response be given an alternative name such as "acceptor" (11). Using this approach, a molecular complex in one target cell would be termed a receptor if a biological response could be demonstrated, while the same complex in the membrane fraction of that cell or on an intact cell of a different type would be termed an acceptor if no biological response could be demonstrated. This type of semantic distinction between receptor and acceptor is highly arbitrary and strongly biased by the limitations of the experiments. Insulin receptors, for example, have now been studied in some detail in intact cells, particulate fractions, and solubilized preparations of at least 10 different tissues. Some of these tissues are classical target tissues for insulin action, while others are tissues not thought to be insulin responsive. In all cases, the major characteristics of the binding reaction including kinetics, pH optimum, neg-

ative cooperativity, and analogue specificity are virtually identical (50); furthermore, anti-insulin receptor antibodies block insulin binding in all of these (37). Thus, by all criteria, these binding sites appear to be the same or nearly the same molecules.

To avoid such semantic confusion, it seems more useful to define a hormone receptor as "a cellular component which has the ability to selectively recognize and bind the hormone and which has binding characteristics consistent with a potential for signal generation." Most importantly, the binding should show specificity for hormonal analogues which parallels known biological effects of these hormones. In addition, the kinetics of hormone-receptor interaction and the affinity of the receptor for the hormone should be consistent with the kinetics and dose response of biological action of the hormone. The biological response, on the other hand, can be regarded as being generated by a functionally separate unit, the effector. The receptor and effector functions may reside in the same single molecule, or more likely, reside in separate molecules which interact directly or indirectly in the fluid mosaic membrane (Fig. 1). Relatively large variations may be observed between hormone binding and biological response depending on the number of steps between these two processes and the manner in which the steps are coupled (see sections VIII and IX). In the most extreme situation, the cell could possess a normal receptor molecule in the total absence of the effector molecules. In this case, no biological response would be observed despite the normal receptor.

### IV. CHEMICAL NATURE OF HORMONE RECEPTORS

No hormone receptor has been isolated in sufficient quality and quantity for complete chemical characterization. There has been considerable progress in our understanding of the chemical nature of receptors, however, derived from a variety of indirect studies such as effects of enzymatic degradation on hormone binding and from attempts to isolate and purify these membrane-bound components (Table I).

All of the hormone receptors that have been tested are susceptible, to some extent, to degradation by one or more proteolytic enzymes, suggesting that in each receptor there is a protein component important for hormone recognition (73, 88, 106, 127). Important roles for membrane phospholipids have been suggested for the receptors

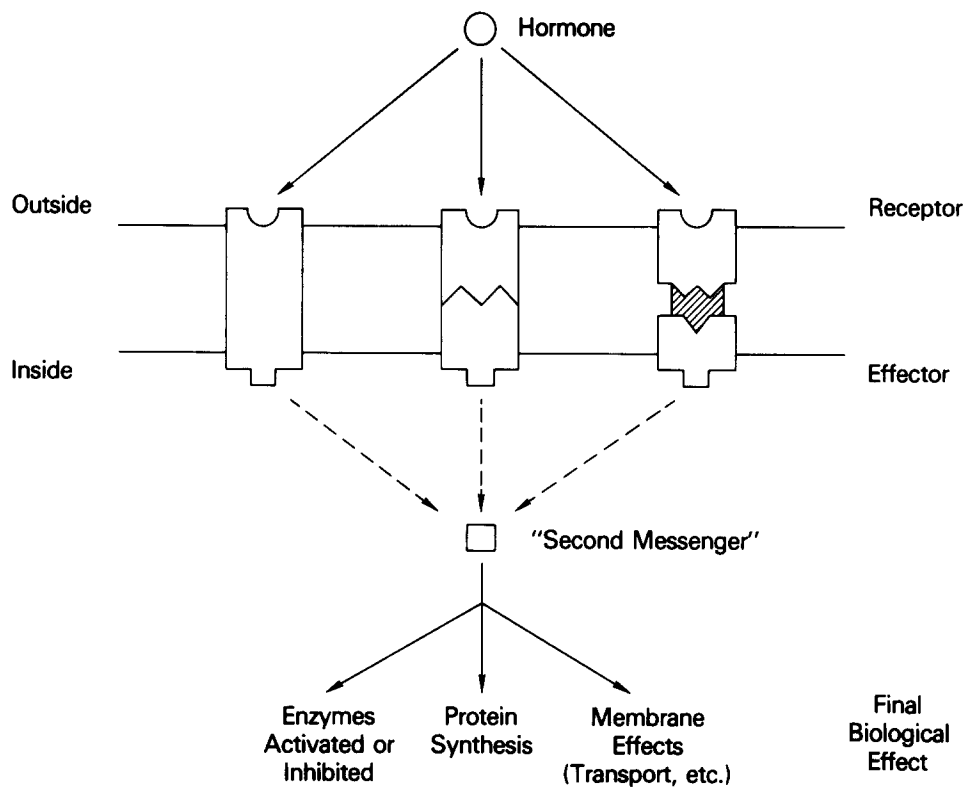


FIGURE 1 Models of hormone receptors and effectors. Three simplified models of hormone receptors and effectors are depicted. In the complex at the left, both the receptor and effector functions are present in a single transmembrane molecule. In the complex at the center, the receptor and effector are separate molecules but directly coupled. In the complex at the right, the receptor and effector are separate molecules and coupled by a third (shaded) molecule.

for adrenocorticotropin (ACTH),<sup>2</sup> glucagon, thyrotropin releasing hormone (TRH), luteinizing hormone-human chorionic gonadotropin (LH-hCG), and both the adrenergic and cholinergic neurotransmitters, all of which exhibit decreased hormone binding after phospholipase treatment (4, 73, 106, 114). The receptors for insulin, thyrotropin (TSH), and the gonadotropins (LH-hCG) appear to have important carbohydrate components. The TSH receptor loses binding activity when exposed to neuraminidase and stains for both protein and carbohydrate on polyacrylamide gel electrophoresis (146). Insulin receptors and

<sup>2</sup> *Abbreviations used in this paper:* ACTH, adrenocorticotropin; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; MSA, multiplication-stimulating activity; NSILA-s, non-suppressible insulin-like activity soluble in acid-ethanol; TRH, thyrotropin releasing hormone; TSH, thyrotropin; and VIP, vasoactive intestinal polypeptide.

gonadotropin receptors are unaffected by neuraminidase alone (23, 32), but the insulin receptor does lose binding activity after exposure to a combination of neuraminidase and  $\beta$ -galactosidase (22). In addition, soluble preparations of all three types of receptors are bound by concanavalin A-Sepharose (24, 119, 146), although in the case of the insulin receptor this interaction appears to be through a site which is separate from the bioactive sites (26). Disulfide exchange has not been proven necessary for any hormone-receptor interaction; however, some receptors appear to have sulfhydryl groups in or near the active site as judged by susceptibility to *p*-chloromercuribenzoic acid or to *N*-ethyl-maleimide (34, 78).

Estimated molecular weights of receptors which have been solubilized in the absence of denaturants have ranged from 140,000 to 550,000 (Table I). Most solubilized peptide hormone receptors have had estimated mol wt of about 200,000

TABLE I  
Chemical Characteristics of Some Hormone Receptors

Receptor	Probable chemical nature	Sulfhydryl dependent	Estimated mol wt	Evidence for subunits	Estimated mol wt of subunit	References
$\beta$ -adrenergic	Lipoprotein	Yes	140,000-160,000	?	—	87, footnote 3
Cholinergic	Glycolipoprotein	Yes	230,000-550,000	Yes	70,000-90,000 (+ smaller)	68, 78, 81, 98, 106, 122, 133
Gonadotropin (LH-hCG)	Glycolipoprotein	Yes	194,000	Yes	90,000	32-34
Glucagon	Lipoprotein	—	190,000	Yes	90,000	52, 80
Insulin	Glycoprotein	No	300,000	Yes	75,000	21-24, 48, 51
Prolactin	Protein	—	220,000	No	—	136
TSH	Glycoprotein	—	280,000	Yes	166,000, 75,000, 24,000	146

(22, 32, 48, 52, 81, 136, 146). In some of these preparations, it has been possible to demonstrate adenylate cyclase activity indicating the complexity of these solubilized preparations and suggesting the close association of the receptor and cyclase (32). Density gradient centrifugation has shown that most solubilized receptor complexes have a relatively low sedimentation coefficient, a high Stokes' radius, and a high axial ratio. Since integral membrane proteins such as receptors generally bind large amounts of Triton X-100 or other nonionic detergents when solubilized (0.2-1.4 mg per mg protein), the very similar features of most solubilized receptors may be the result of the high partial specific volume of the complex due to bound detergent and to the asymmetry and abnormal hydration of these complexes (62, 98).

Attempts to purify and chemically characterize hormone receptor have proceeded slowly. This is not surprising in view of the small number of molecules per cell and the relative insolubility of these macromolecules. If one begins with a crude microsomal fraction which contains 0.2 pmol of receptor per milligram of protein and assumes that the receptor has only one binding site per 200,000 daltons, complete purification (to a sp act of 5,000 pmol/mg protein) will require about a 25,000-fold enrichment. The density of acetylcholine receptors in electric tissue from *Torpedo* or electric eel is 500- to 5,000-fold higher, making purification somewhat simpler (26).

Purification of the acetylcholine receptor from electric tissue of fish has been pursued in several laboratories. When extracted with Triton X-100 or other nonionic detergents, the acetylcholine receptor has a mol wt of between 250,000 and 550,000 daltons (17, 68, 98, 106, 119). Purifica-

tion has yielded milligram quantities of receptor (19, 68, 78, 81, 99, 119, 133). These highly purified preparations have one neurotoxin binding site per 70,000-90,000 daltons (6-13 nmol per mg protein) (107, 157) and 5-6% carbohydrate (119), suggesting that the native receptor is an oligomeric glycoprotein. On electron micrographs, the purified receptor appears as a ringlike particle 8-9 nm in diam consisting of five to six subunits, each 3-4 nm in diam, surrounding an electron-dense pit (19). Several different protein unit patterns have been observed on polyacrylamide gel electrophoresis in SDS. The acetylcholine receptor of the eel consists of two major types of subunits, one of 42,000 and another of 54,000 mol wt (68, 122) while that from *Torpedo* contains three of four major bands on SDS-polyacrylamide electrophoresis varying in mol wt from 26,000 to 64,000 (133, 152). Since these components no longer retain the acetylcholine-binding property, it is not clear which of these compose the actual active binding site of the receptor.

Amino acid analysis of the acetylcholine receptors from four different species reveals a relatively high content of proline, aromatic amino acids, and polar amino acids and a low degree of hydrophobicity (101). UV circular dichroism spectroscopy of receptor from *Torpedo* also has revealed a high content of ordered secondary structure (34%  $\alpha$  helix and 27%  $\beta$  structure). Although, the amino acid composition and protein subunit structures are different in the acetylcholine receptors examined, there has been a significant preservation of structure. Furthermore, immunization of rabbits with receptors from the electric eel produces a myasthenia gravis-like illness suggesting that the acetylcholine receptors in these two species possess similar immunologic determinants (112).

The most extensively characterized and purified

<sup>3</sup> Lefkowitz, R. J. Personal communication.

preparation of a peptide hormone receptor is that for the gonadotropins LH and hCG which has been prepared by Dufau and her co-workers (32, 33). When gonadal membranes were solubilized with Lubrol PX (ICI America Inc., Stamford, Conn.), two major peaks of hormone binding were observed. The first peak had an apparent mol wt of about 200,000 and was coincident with the adenylate cyclase activity, while the second peak, with an approx. mol wt of 80,000, appeared to represent receptors which had been dissociated from a receptor-cyclase complex (32). Using affinity chromatography, Dufau et al. have purified the gonadotropin receptor to a sp act of 2,500 pmol/mg protein (33). This highly purified receptor retains its affinity and specificity for gonadotropins, and is a single component on gel electrophoresis in SDS with an approx. mol wt of 90,000. This preparation appears to be about 25% pure.

Other peptide hormone receptors have been purified in only very small quantity or to lower specific activity. These include the receptors for insulin (21), glucagon (52), and prolactin (13) (Table I). It is interesting to note that the solubilized prolactin receptor has a higher affinity for hormone than the native receptor.

Like the acetylcholine receptor, the receptors for the polypeptide hormones may be oligomeric complexes. For several of the soluble receptor preparations, it has been possible to demonstrate a smaller subunit which possesses the binding property of the whole receptor complex. In the case of the glucagon receptor, a 20,000-dalton molecule which binds glucagon has been detected after the larger soluble receptor is exposed to a high concentration of glucagon (80). Similarly, an active 24,000-dalton fragment of the TSH receptor has been produced by limited proteolysis (146). Preliminary studies in this laboratory also suggest that the soluble insulin receptor, which has a mol wt of about 300,000, is an oligomeric structure probably comprised of four or more binding sites (51).

## V. THE BINDING PROPERTIES OF HORMONE RECEPTORS

The binding of labeled hormones to their receptors has demonstrated several characteristics which have helped to form part of the functional definition used for identifying receptors for the peptide hormones and neurotransmitters.

### 1. Hormone Binding to its Receptor is Rapid and Usually Reversible

The time course of hormone binding to its receptor is dependent on temperature and on the concentrations of hormone and receptor. At 37°C steady states are observed within a few minutes, while at lower temperatures a steady state may not be reached for hours (11, 12, 14, 38, 41, 47, 56, 60, 74, 88, 96, 119, 127, 140, 151) (Fig. 2). With

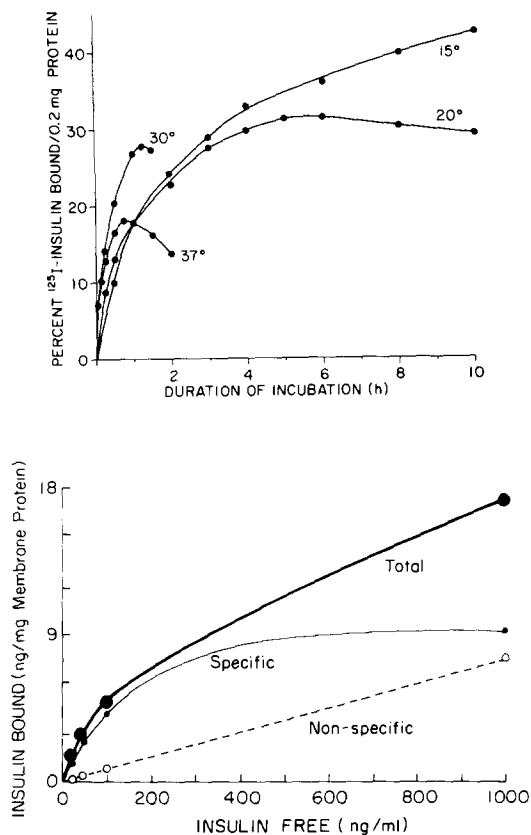


FIGURE 2 Time-course and saturability of hormone binding to receptors. (Top) Time-course of insulin binding to liver plasma membranes as a function of temperature. Mouse liver plasma membranes (0.2 mg protein/ml) were incubated with <sup>125</sup>I-insulin (20 pM) at the indicated temperatures. The percent of <sup>125</sup>I-insulin specifically bound is plotted as a function of time (adapted from reference 140). (Bottom) Insulin bound as a function of insulin concentration. Insulin binding to rat liver plasma membranes was determined as a function of insulin concentration by adding increasing amounts of unlabeled insulin to a tracer of <sup>125</sup>I-insulin. The data are expressed as total, specific, and nonspecific insulin bound as a function of free insulin concentration.

the exception of the  $\beta$ -adrenergic receptor (12), association rate constants which have been determined for the hormone receptor interactions have been of the order  $10^4$ - $10^7$   $M^{-1} s^{-1}$  (Table II). These rates are significantly lower than would be predicted for a simple diffusion limited process; for example, a hormone of 6,000 mol wt interacting with a 150,000-dalton receptor would be expected to have a forward rate constant of  $6 \times 10^8$   $M^{-1} s^{-1}$  (74). This suggests that most random collisions do not result in a binding reaction and that the reacting species almost certainly have to be preferentially aligned or overcome an activation energy. Addition of an excess of unlabeled hormone, dilution of the reaction mixture or changing pH results in a rapid dissociation of the bound tracer (Table II). The hormone dissociated from the receptor appears to be identical with the native hormone with respect to its physical properties, interaction with antibodies or other membrane receptors, and biological activity (32, 42, 88, 127).

## 2. There is a Finite Number of Receptor Sites on the Cell

The quantitative aspects of the hormone-receptor interaction are complex. In all systems, binding has been considered to be the sum of at least two processes, one of which is saturable and has been termed "specific" binding, and the other of which is not saturable over the range of concentrations studied and has been termed "nonspecific" (Fig. 2). The latter is composed of low affinity binding to the membrane, as well as nonspecific trapping and adsorption to the glass or plastic incubation tube. Whether binding of hormones to these low affinity nonspecific membrane sites plays any role in hormone action is uncertain. In studies of lac repressor binding to DNA, it has been suggested that binding to the nonspecific sites increases the rate of repressor binding to operator and is important for the regulation of the lac operon (95). In any case, in the physiological range, most binding occurs to the specific receptor sites, which by definition are finite in number. The actual number of specific receptor sites for the peptide hormones and catecholamines has ranged from as low as 500 to as high as 250,000 sites per cell (Table II). Acetylcholine receptors have been estimated to exist in specialized electric tissue cells at concentrations in excess of  $10^{11}$  per cell (19).

## 3. Hormone Receptors Have a High Affinity for the Hormone

A variety of mathematical methods have been applied to the analysis of steady-state binding data in an attempt to derive affinity constants for the hormone receptor interaction (for a complete review of the methods and their assumptions and limitations, see references 11, 26, 73, 123, and 124). In most of these analyses, the hormone-receptor interaction is depicted as a simple reversible bimolecular equilibrium,



and

$$\frac{k_a}{k_d} = \frac{[HR]}{[H][R]} = K \quad (2)$$

where  $[H]$  is the concentration of free hormone,  $[R]$  is the concentration of unoccupied receptors,  $[HR]$  is the concentration of hormone-receptor complexes,  $k_a$  and  $k_d$  are the association and dissociation rate constants, and  $K$  is the affinity or equilibrium constant for the reaction. This type of simple bimolecular reaction between a hormone and its receptor appears to be present for growth hormone (90), calcitonin (96), prolactin (136), the gonadotropins LH-hCG (32, 121), and, in some tissues, for the cholinergic (81, 119) or  $\beta$ -adrenergic receptors (2, 14). In these, the apparent equilibrium constants for the binding reaction derived from steady-state data have ranged from  $5 \times 10^5$   $M^{-1}$  for the interaction of acetylcholine with the cholinergic receptor to  $5 \times 10^{10}$   $M^{-1}$  for the interaction of the  $\beta$ -antagonist hydroxybenzylpindolol with the  $\beta$ -adrenergic receptor (Table II). Most peptide hormone receptors have affinity constants of  $10^8$ - $10^{10}$   $M^{-1}$ . In general, similar values have been obtained from kinetic experiments.

In many cases, the reaction appears to be more complex. Several hormones have equilibrium binding data which suggest that there are two or more classes or orders of binding sites which differ in affinity for the hormone. This has been reported for receptors for ACTH (88), oxytocin (70), glucagon (137), TRH (60), TSH (102), insulin (74), acetylcholine (96, 107), and catecholamines (89). In these, analysis of equilibrium and kinetic data has been more difficult.

Recently, De Meyts and his co-workers (26-29)

TABLE II  
Binding Properties of Some Selected Hormone and Neurotransmitter Receptors

Receptor	Tissue	No. of sites/cell	Cooperative site-site interactions	Equilibrium binding constants (M <sup>-1</sup> )	Rate constants			References
					Association (s <sup>-1</sup> M <sup>-1</sup> )	Dissociation (s <sup>-1</sup> )	References	
β-Adrenergic	Frog erythrocyte	1,300-1,800	Negative	~10 <sup>8</sup> *	1 × 10 <sup>8</sup>	2.6 × 10 <sup>-3</sup>	94, 103	
	Turkey erythrocyte	400-600	None	5 × 10 <sup>7</sup> ‡			2, 14	
Cholinergic	Electric tissue of eel	>1,000,000	None	9 × 10 <sup>8</sup> (α-toxin) 5 × 10 <sup>8</sup> (acetylcholine)	1.35 × 10 <sup>7</sup>	1.52 × 10 <sup>-4</sup>	19, 81 119	
Angiotensin	Adrenal		None	0.2-0.5 × 10 <sup>8</sup>	2.4 × 10 <sup>5</sup>	5 × 10 <sup>-4</sup>	56	
Glucagon	Liver	110,000	?	~1.5 × 10 <sup>8</sup>	1 × 10 <sup>6</sup>	4 × 10 <sup>-3</sup>	41, 127	
LH-hCG	Leydig cell	6,000	None	0.5-1 × 10 <sup>10</sup> §	1 × 10 <sup>4</sup>	2 × 10 <sup>-5</sup>	15, 32	
	Ovary		None	0.6 × 10 <sup>10</sup> §	2.8 × 10 <sup>8</sup>	2.1 × 10 <sup>-3</sup>	121	
Growth hormone	Lymphocyte	4,000	None	1.3 × 10 <sup>8</sup>			90	
Insulin	Liver	100,000-250,000	Negative	1.2 × 10 <sup>8</sup>	0.13-1 × 10 <sup>6</sup>	0.7 × 10 <sup>-3</sup>	26, 27, 41, 74, 140	
	Adipocyte	50,000	Negative§	3.3 × 10 <sup>8</sup>	4.1 × 10 <sup>6</sup>	1.1 × 10 <sup>-3</sup>	47, 54, 82	
	Monocyte	16,000	Negative	1.3 × 10 <sup>8</sup>			26-29	
	Turkey erythrocyte	3,000	Negative	3.2 × 10 <sup>8</sup>			50, 51	
Nerve growth factor	Nerve		Negative	2 × 10 <sup>10</sup> ¶ to 5 × 10 <sup>8</sup>	7.5 × 10 <sup>6</sup>	3.8 × 10 <sup>-4</sup> to 1.2 × 10 <sup>-2</sup>	38	
TSH	Thyroid	~500	Negative	1.9 × 10 <sup>8</sup>	1 × 10 <sup>7</sup>	6 × 10 <sup>-3</sup>	146, 151, 153	
Vasopressin	Kidney		Positive	~5 × 10 <sup>8</sup>	3.8 × 10 <sup>5</sup>	7.5 × 10 <sup>-4</sup>	12, 70, 120	

\* Determined for the β-adrenergic antagonist (-) Alprenolol. The affinity for β-adrenergic agonists is lower.  
 † Determined with the β-adrenergic antagonist hydroxybenzylpindolol. The affinity of β-adrenergic agonists is lower.  
 § Kahn, C. R., and K. Baird. Unpublished observation.  
 || Affinity of the high affinity (empty conformation) of the receptor (see reference 10).  
 ¶ The two values represent the high and low affinity conformations, respectively.



and Levitzki (92) have suggested that the complex binding isotherms in many hormone-receptor interactions may be the result of site-site interactions or cooperativity among receptors. The reaction is considered positively cooperative if the binding of the first ligand increases the affinity of the receptor for the second and negatively cooperative if the binding of the first ligand decreases the affinity of the receptor for the second (26).

Cooperativity in hormone binding to receptors appears to be a frequent finding. Homotropic (hormone-induced) positive cooperativity has been suggested in some studies of the vasopressin-receptor interaction (12, 70) and the acetylcholine-receptor interaction (35). Evidence for homotropic negative cooperativity, on the other hand, has been presented in studies of receptors for insulin (27, 29, 50), TSH (151),<sup>4</sup> TRH (60), nerve growth factor (38), and in some studies of  $\beta$ -adrenergic (94) and cholinergic (35) receptors. The decrease in receptor affinity observed at high receptor occupancy in most cases of negative cooperativity appears to be due to an increase in the rate of dissociation of the hormone-receptor complex. These kinetic findings are important since, from steady-state analysis alone, it is impossible to distinguish negative cooperativity from site heterogeneity (29). Cooperative effects are retained in solubilized insulin (51) and TSH (72) receptors, and in a tryptic fragment of the TSH receptor (146). In the case of the insulin receptor, the negative cooperative interaction is associated with a decrease in size of the solubilized receptor (51), perhaps due to subunit dissociation, and results in about a 10-fold fall in receptor affinity (27, 28, 50). In addition, regions distinct from the bioactive site of the insulin and insulin receptor molecules appear to be required for the cooperative effect (28). These findings suggest that some receptors can exist in at least two reversible conformations: a "high affinity" conformation from which the labeled hormone dissociates slowly and which is present at low occupancy, and a "low affinity" conformation present at high occupancy from which labeled hormone dissociates rapidly (27, 28).

The affinity of a hormone for a receptor may also be altered by binding of a different ligand to the receptor (heterotropic cooperativity). This reaction has been most extensively studied with regard to the effect of the guanyl nucleotides on the

glucagon (128, 142) and angiotensin receptors (55). In both of these, GTP stimulates the dissociation of these hormones from their respective receptors. Profound effects of guanyl nucleotides have been also observed in the action of several of the peptide hormones and catecholamines, suggesting that the guanyl nucleotides may act in concert with the hormone receptor as an allosteric regulator of biological response (10, 128). Divalent cation concentration and pH also have profound effects on binding of some hormones, suggesting that these too may act as heterotropic regulators of receptor affinity.

Several possible mechanisms to explain these various cooperative effects have been proposed. The most likely include conformational changes in the structure of oligomeric receptors, or polymerization (clustering) or depolymerization of receptors in the fluid membrane, or a combination of these (26-29, 92).

#### *4. The Binding Sites are Specific for the Hormone and Binding of the Hormone to the Sites can be Related to the Biological Effects of the Hormone*

This is the major characteristic and *essence* of the functional definition of the hormone receptor. A single hormone receptor binds only one type of hormone and binding of the hormone to this site can be correlated with biological activity of the hormone. In practice, these properties are usually demonstrated by showing that there is competition for binding of labeled hormone to the receptor only by unlabeled substances which are related to the hormone in question, and more specifically, that the relative potency of hormone analogues to compete for the binding sites is in direct proportion to their bioactivities. Among receptors for peptide hormones, this property has been most extensively studied for the insulin receptor by Freychet et al. (39, 44) and Gliemann and Gammeltoft (53). These investigators have studied over 40 insulin analogues, and, in every case, have shown a close correlation between affinity of the analogue for the insulin receptor and its biological activity (Fig. 3). Similar data exist for the vasopressin receptor (12, 70), the  $\beta$ -adrenergic receptor (2, 14, 103), and the cholinergic receptor (19, 106, 119) and to a lesser extent for other peptide hormone receptors. This high degree of biological

<sup>4</sup> Kohn, L. Personal communication.

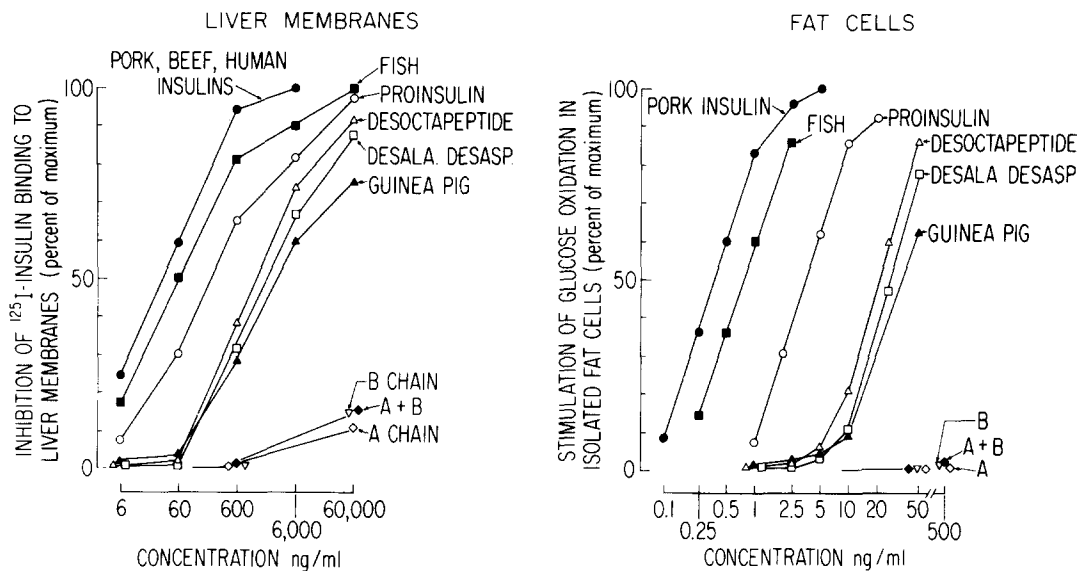


FIGURE 3 Effect of insulin and insulin analogues on  $^{125}\text{I}$ -insulin binding to liver membranes and glucose oxidation in fat cells. (Left) Inhibition of  $^{125}\text{I}$ -insulin binding to rat liver membranes expressed as percent of maximum is plotted as a function of the concentration of unlabeled peptide. (Right) The stimulation of glucose oxidation by isolated rat fat cells expressed as percent of maximum is plotted as a function of the concentration of unlabeled peptide. Note the close correlation between affinity for the receptor (left) and biological effect (right). From Freychet et al. (44); used with permission.

specificity is in marked contrast to the specificity demonstrated by antibody-binding sites and has allowed hormone-receptor studies to be used to map the structure-function relationships of hormone molecules (65, 70, 116, 126) and also has been applied to the assay of plasma hormones (77). True competitive antagonists of hormone action compete for the binding site but do not initiate the biological response (11, 103, 126).

Hormones with similar biological activities may or may not share the same receptor. LH and hCG, for example, appear to act directly on the same receptor site in the gonad (32, 121), while the closely related hormones secretin, glucagon, and vasoactive intestinal polypeptide appear to have separate receptor sites (31, 126, 127). An intermediate case is that of enteroglucagon which can bind to some, but not all, of the hepatic receptors for pancreatic glucagon (5).

Hormones with some spectrum of overlapping biological activities present more complicated pictures. Growth hormone, prolactin, and placental lactogen appear to act through at least two receptor sites, one more specific for the growth hormone aspect of these peptides (90) and the other more specific for the lactogenic aspects of these peptides (136). Similarly, insulin has an overlap-

ping spectrum of activities with several serum growth factors (nonsuppressible insulin-like activity soluble in acid-ethanol [NSILA-s], multiplication-stimulating activity [MSA], and the somatomedins) (149). Again, these activities appear to be mediated by one receptor which has a high affinity for insulin and a lower affinity for the growth factors which mediates the acute metabolic effects of these peptides and one or more receptors which have a higher affinity for the growth factors than for insulin which are thought to mediate the growth factors of these peptides (97, 149).

## VI. SUBCELLULAR LOCALIZATION OF RECEPTORS

Receptors for peptide hormones and neurotransmitters are in their highest concentration on the plasma membrane of the cell. This finding is supported by direct studies of labeled hormone binding to intact cells (15, 39, 41, 55, 82, 153) and by the finding that the receptor concentration is enriched 30–100-fold during purification of the plasma membrane (44, 73, 105). Several investigators, however, have found binding sites for peptide hormones and catecholamines on intracellular organelles (10, 72, 75). Fractions of both smooth

and rough endoplasmic reticulum, Golgi apparatus, and even nuclei<sup>5</sup> all have hormone binding sites (Fig. 4, and references 9, 66). Some of these sites have an affinity and specificity for hormone analogues similar to that of the plasma membrane receptor, while others do not (bottom of Fig. 4). Whether these are true receptors in the process of synthesis or degradation or some other type of hormone-binding sites remains to be determined. Awareness of these nonplasma membrane sites is important when studying crude particulate fractions of cells, since in these fractions intracellular organelles may contribute a larger fraction of protein than the plasma membrane (105).

On the plasma membrane itself, receptors may not be homogeneously distributed. The most extreme example of this is the cholinergic receptors which have been estimated by immunofluorescence and autoradiography studies to be heavily clustered in subsynaptic areas (10,000–100,000 per  $\mu\text{m}^2$ ) while having only 0.01 this density outside of the synapses (13, 19). Peptide hormones may also show some degree of polarization or clustering of receptors. Using ferritin-labeled insulin, Orci et al. (110) and Jarett and Smith (71) have studied the distribution of insulin receptors on liver and fat cell membranes. In both cases, the insulin-binding sites were observed at a density of about 90 receptors per  $\mu\text{m}^2$ . In some areas, the receptors were diffusely distributed while in other areas clusters containing 3–12 ferritin insulin molecules were seen (Fig. 5). The receptors appeared to be associated with the glycocalyx of the adipocytes (71), and in freeze-etched preparations of liver membranes the binding sites corresponded to intramembrane particles which could be seen embedded in the phospholipid bilayer (110). No receptors were found on the inside of the adipocytes or liver membranes by this technique or could be found by direct study of inverted membrane vesicles prepared from adipocytes (7), suggesting that the receptors face only the external surface of the plasma membrane.

In almost all systems, the major site of hormone degradation appears to be separate from the hormone receptor site. This has been most clearly demonstrated in the case of acetylcholine-esterase and the acetylcholine receptor which can be physically separated by gel filtration or density gradient fraction in the presence of detergents (19, 106). With peptide hormones and catecholamines, the

separation of these two active sites has been inferred from differences in specificity, affinity, optimal conditions for binding vs. degradation and effects of enzyme treatment, temperature, and specific inhibitors (30, 42, 106). While hormone-degrading enzymes can be demonstrated in purified plasma membrane fractions of the cell, the total activity is usually greater in less purified fractions, suggesting that some hormone degradation may take place intracellularly (41).

## VII. REGULATION OF HORMONE RECEPTOR CONCENTRATION

Like other components of the cell, the constituents of the plasma membrane are in a state of continual turnover. Membrane proteins have an average half-life of 30–60 h, with a range of 2 h to 16 days for various specific membrane-associated enzymes (132). The concentration of hormone receptors, like that of other membrane components, is not fixed, but is a reflection of a state of continual synthesis and degradation. Any change in the rate of synthesis or degradation will result in a change in the concentration of hormone receptor. Over the past 3–4 yr, a variety of studies have suggested that changes in plasma membrane receptor concentrations provide a potential mechanism for regulation of target cell sensitivity to the hormone.

At present, very little is known about normal rates of receptor turnover. Studying rat diaphragm in tissue culture by a prelabeling technique, Berg and Hall (8) found that only 20% of acetylcholine receptors were lost per 24 h from innervated muscle and that this loss increased to 80% per 24 h when the muscle was denervated. Inhibitors of energy and protein synthesis decreased the rate of loss. In cultured human lymphocytes, turnover of growth hormone and insulin receptors has been estimated by blocking synthesis with cycloheximide; growth hormone receptors have a  $t_{1/2}$  of about 10 h (91) while insulin receptors have a  $t_{1/2}$  of 30–40 h.<sup>6</sup>

A variety of factors regulate the concentration of receptors. One major factor is the hormone itself. Data from a variety of animal and human disease models have demonstrated an inverse relationship between the serum concentration of a hormone and the concentration of its membrane receptors. In the very hyperinsulinemic and insulin-resistant obese-hyperglycemic mouse, there is

<sup>5</sup> Goldfine, I. D. Personal communication.

<sup>6</sup> Kosmakos, F., and Roth, J. Manuscript in preparation.

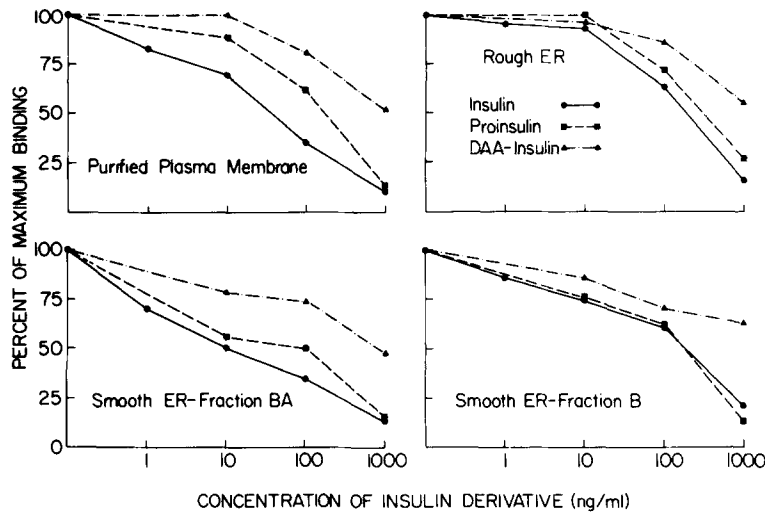
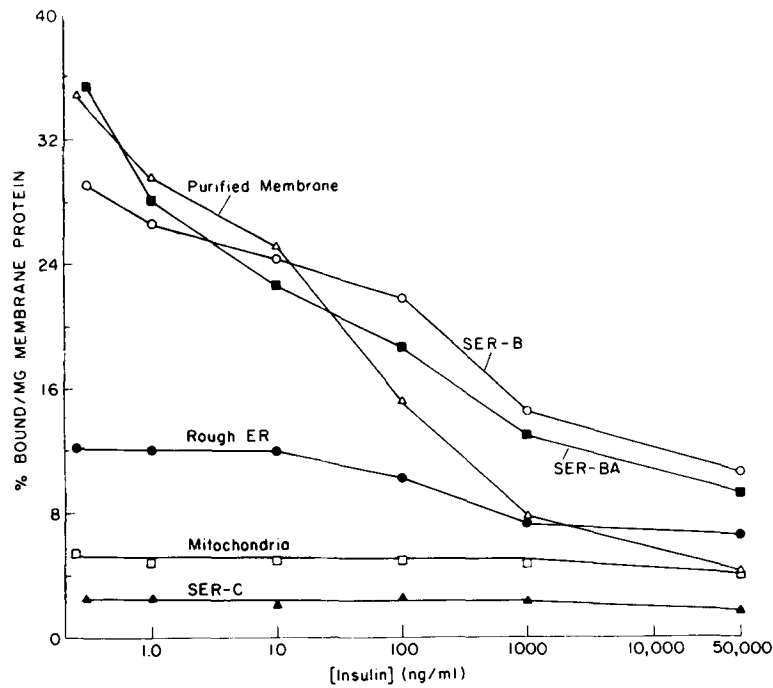


FIGURE 4 Insulin binding to subcellular fractions of liver. (Top) Rat livers were homogenized in 1 mM  $\text{NaHCO}_3$  and subcellular fractions separated by isopycnic centrifugation in sucrose density gradients. Three fractions of smooth endoplasmic reticulum of increasing density (SER BA, SER B, and SER C), a fraction of rough endoplasmic reticulum, a mitochondrial fraction and the purified plasma membrane were obtained. Fractions were then incubated with  $^{125}\text{I}$ -insulin (30 pM) and increasing concentrations of unlabeled insulin for 60 min at  $30^\circ\text{C}$ . The percent  $^{125}\text{I}$ -insulin bound is plotted as a function of total insulin concentration. Note that SER BA and SER B show significant high affinity insulin binding, while the rough ER has insulin binding sites of lower affinity. (Bottom) Fractions as described above were incubated with  $^{125}\text{I}$ -insulin and increasing concentrations of insulin, proinsulin, and desalanine desasparagine insulin (DAA-insulin). Note that in the purified plasma membrane these compete for insulin binding in direct proportion to their bioactivity (see Fig. 3). In the other fractions, particularly SER B, the binding sites have less ability to distinguish these analogues. C. R. Kahn and D. M. Neville, Jr., unpublished observations.

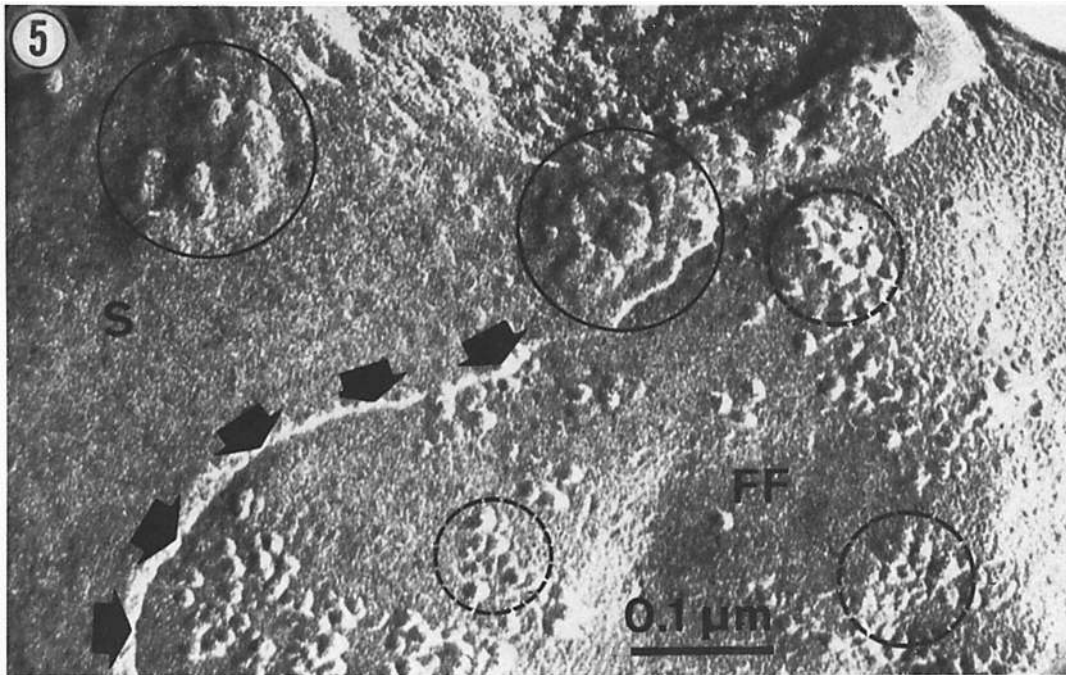


FIGURE 5 Ferritin-insulin binding to liver plasma membranes as viewed in the electron microscope. Purified liver plasma membranes have been split by freeze-fracturing to expose the inner matrix containing intramembranous particles (*FF*, fracture face), and deep-etched to expose the outer surface (*S*) of the membrane. The outer surface of the membrane shows clusters of ferritin-insulin molecules (enclosed by the solid circles) which appear similar to the clusters of intramembranous particles on the fracture face (enclosed by the broken circles). The arrows mark the ridge which separates the outer surface from the fracture face.  $\times 90,000$ . Courtesy of L. Orci et al. (110).

a 50–70% decrease in the number of insulin receptors on hepatocytes (75, 76, 140), adipocytes (43), thymocytes (139), and cardiac muscle (40) (Fig. 6). The receptors which remain appear to be normal in their affinity for insulin, kinetics of association and dissociation, temperature dependence of binding, biological specificity, and negative cooperative interactions (140). Similar decreases have been observed in other genetically obese and insulin-resistant mice (6, 141), in normal mice made obese and insulin resistant by administration of gold thioglucose (141), and in rats made insulin resistant by administration of steroids or implantation of pituitary tumors which secrete ACTH and  $\text{GH}_1$  (59, 108). Furthermore, insulin receptors on circulating mononuclear leukocytes from a hyperinsulinemic thin or obese man are decreased and the magnitude of the decrease correlates well with the degree of hyperinsulinemia (1, 109). Conversely, insulin-deficient states in animals are associated with an increase in the concentration of insulin receptors on the cell

membrane (57, 63, 141). In each case, when the cause of the altered insulin concentration is corrected, e.g., dieting the obese animals, the insulin receptor concentration returns toward normal.

This alteration in insulin receptors is a specific membrane alteration. In the obese hyperglycemic mouse which has been most extensively studied (75, 76, 140, 141), fully purified plasma membrane fractions of liver of the *ob/ob* mouse demonstrate a 70% decrease in insulin receptors but remain identical to those of the thin littermates with respect to morphologic appearance, activities of 5'-nucleotidase, and adenylate cyclase, and protein subunit composition. In addition, the membrane receptors for glucagon, growth hormone, and isoproterenol are not altered in these animals (Fig. 6). Chang et al. (16) have suggested that the decrease in insulin receptors is a part of a more generalized alteration in membrane glycoproteins since lectin binding to crude membrane fractions and enzymatically treated cells is also decreased. This interpretation does not seem war-

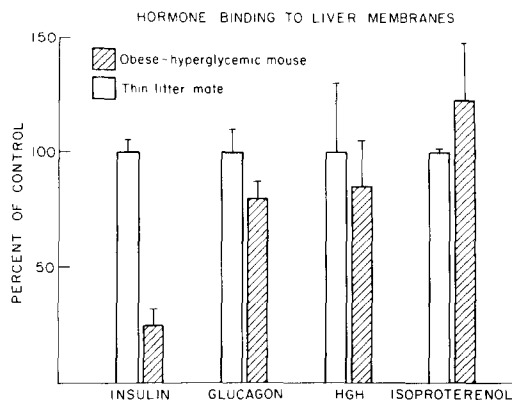


FIGURE 6 Binding of  $^{125}\text{I}$ -insulin,  $^{125}\text{I}$ -glucagon,  $^{125}\text{I}$ -human growth hormone (hGH), and  $^3\text{H}$ -isoproterenol to purified liver membranes of obese hyperglycemic mice and their thin littermates. Methods and data are in Kahn et al. (76). All data have been normalized so that the thin littermates (controls) are arbitrarily assigned a value of 100%.

ranted since the cell membrane glycoproteins may certainly have been altered by the enzyme treatment employed. Furthermore, the value of lectin binding as a quantitative marker for plasma membrane glycoproteins in crude particulate fractions, especially in animals with disordered carbohydrate metabolism, is unproven. It is difficult to envision a severe generalized defect in membrane glycoproteins which would have little effect on other aspects of the membrane structure and function.

In vivo a variety of other examples of inverse regulation of a receptor by its hormone have been observed. Parathyroidectomy, which decreases serum calcitonin concentrations, is associated with increased calcitonin binding to membranes of rat kidney (143). This effect can be prevented by administration of exogenous calcitonin. Similarly, denervation of muscle increases acetylcholine receptors and acetylcholine hypersensitivity (36). A decrease in  $\beta$ -adrenergic receptor sites occurs on the erythrocytes of frogs treated with  $\beta$ -adrenergic agonists (104) and on pineal cells of rats treated in vivo with L-isoproterenol or stimulated physiologically by prolonged exposure to dark (79). Both the frog erythrocytes and pineal cells show a decrease in isoproterenol-stimulated adenylate cyclase which parallels the decrease in receptors, while basal and fluoride-stimulated adenylate cyclase remain unchanged. Administration of potent  $\beta$ -adrenergic antagonists does not alter the level of the catecholamine receptor, but may prevent its

modulation by agonists (87).  $\beta$ -adrenergic receptors on rat pineal cells also exhibit a circadian periodicity which is inversely related to the cycle of neurotransmitter release, suggesting that this self regulation of receptors occurs physiologically (79).

Direct effects of hormones on their own receptor sites have also been demonstrated in vitro. Gavin et al. (49) have found a time-dependent loss of insulin receptors when the human lymphocytes were cultured in media containing insulin. With each insulin concentration, a particular rate of loss and steady-state level was achieved (Fig. 7). Analogues of insulin which vary in biopotency and affinity for the insulin receptor exert this regulatory effect in direct proportion to their bioactivities; the effect is also observed with insulin analogues which cannot induce the site-site interactions among insulin receptors, suggesting that the mechanism of regulation and cooperativity are independent.<sup>6</sup> When the cells are returned to medium free of insulin, there is a return of receptors to normal levels within 16 h. Both the "down regulation" of receptors and the return are blocked by cycloheximide.

In the same human lymphocyte line, negative feedback regulation of growth hormone receptors by growth hormone has also been demonstrated (91). This effect can be seen with concentrations of growth hormone as low as  $10^{-10}$  M. This effect is reversed upon removal of the hormone, and the recovery can be blocked by cycloheximide. Growth hormone has no effect on the concentration of insulin receptors, and vice versa.

In vitro negative feedback regulation by hormones of their own receptors has also been observed in studies of TRH receptors in rat pituitary cells (64) and  $\beta$ -adrenergic receptors in frog erythrocytes (100). In both, the ability of analogues to regulate receptor concentration corresponds to their biological activity. Furthermore, the effect on  $\beta$ -adrenergic receptors is produced only by  $\beta$ -adrenergic agonists, is accompanied by a decrease in catecholamine-sensitive adenylate cyclase, and may be blocked by competitive antagonists (87).

Self regulation of membrane receptors is a common phenomenon (118), although the exact mechanism by which it occurs is uncertain. In most cases it appears that binding of the ligand promotes degradation of the receptor by either membrane-associated proteases, endocytosis, or shedding of the hormone-receptor complex into the

## REGULATION OF INSULIN RECEPTORS BY INSULIN

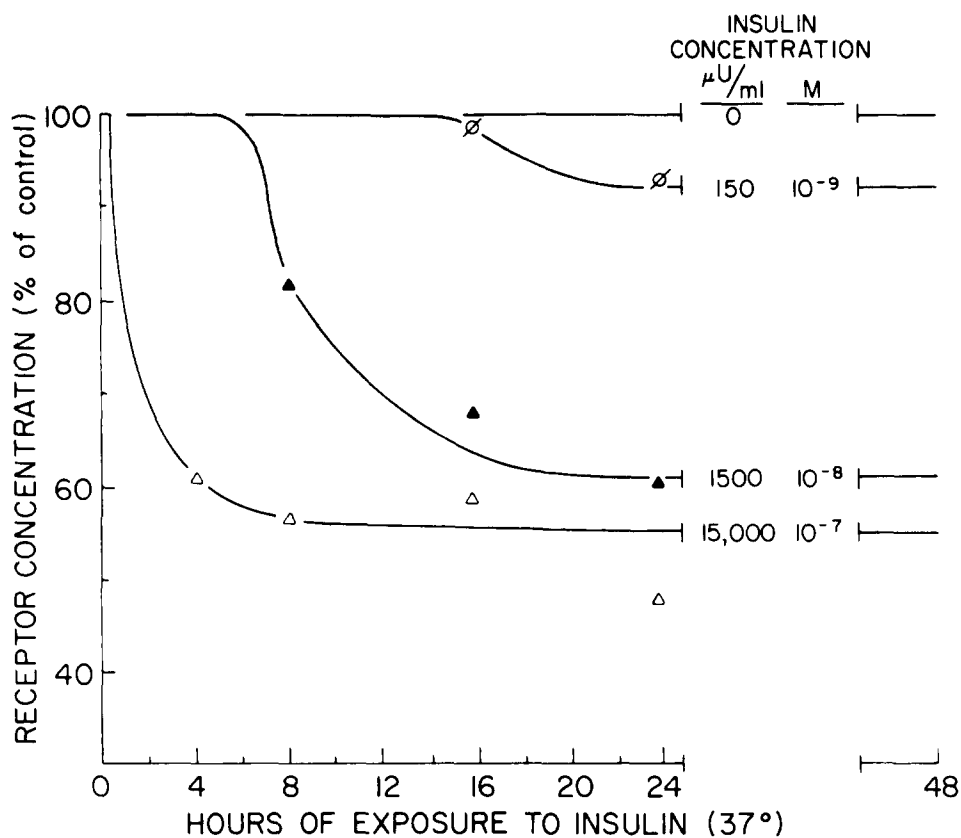


FIGURE 7 Regulation of insulin receptors by insulin. Human lymphoblastoid cells (IM-9) which exist in continuous culture were cultured for up to 48 h in the presence of the indicated insulin concentrations. At the indicated times, cells were extensively washed, and the insulin receptor concentration was determined. Receptor concentration showed a time- and dose-dependent fall in cells which had been exposed to insulin. Modified from Gavin et al. (49) with permission.

medium. The hormone-induced losses of insulin and TRH receptors appear to require protein synthesis (64).<sup>6</sup> Huang and Cuatrecasas have suggested that observed regulation of insulin receptors by insulin is due to intrinsic proteolytic activity of the insulin itself (67). This seems highly unlikely in view of the specificity of the regulatory phenomenon, the close correlation of regulatory potency of a hormone and its biological effect, blockade of the effect in some cases by cycloheximide, and the widespread occurrence of this regulatory mechanism for other types of receptors.

In addition to the negative self-regulation a hormone may exert on its receptors, Posner et al. have presented indirect evidence that the prolactin receptor is under positive feedback regulatory

control by prolactin (115). Heterologous hormones may also influence receptor concentration. Follicle stimulating hormone (FSH) administration to animals appears to result in an increase in the number of LH receptors (or cells with LH receptors) in the ovary (154), while adrenal steroids modulate both the number of vasopressin receptor sites and coupling of the receptor to the hormonal-sensitive adenylate cyclase (120).

Other factors which influence the cell membrane may also influence the concentration of hormone and neurotransmitter receptors on cells. Thomopoulos et al. have found a marked increase in concentration of insulin receptors on fibroblasts as the cells enter a confluent, stationary growth phase (147). Several groups have reported the

appearance of acetylcholine receptors during culture of myogenic cell lines (111, 145). In the latter case, there is also increased clustering during the differentiation of the cell. The most dramatic cell cycle effect is that described by Varga et al. who noted MSH receptors and stimulation of tyrosinase activity by MSH on a melanoma cell line only during the G<sub>2</sub> phase of the cell cycle (150). In each of these cases, the major change appears to be in receptor number, not affinity.

At present, the effect of malignant transformation on the concentration of hormone receptors concentrations has been investigated only for insulin receptors. Thomopoulos et al. have found that the concentration of insulin receptors on BALB/3T3 fibroblasts transformed by a variety of viruses, X ray, methylcholanthrene or spontaneously is low and in the same range as that of rapidly growing normal cells (147). Insulin binding increases as the transformed cells reached higher densities in culture, but not to the levels seen in normal cells. In contrast, Krug et al. reported an increase in the concentration of insulin receptor on lymphocytes after activation by concanavalin A (85). However, the dose of concanavalin A used was toxic to a large percentage of the cells and much of the insulin binding observed showed poor displacement by unlabeled insulin, suggesting a low-affinity type of binding. Rabinowitz et al. found no increase in insulin receptors on lymphocytes transformed with a low, nontoxic dose of concanavalin A (117).

Three points regarding all of the studies of receptor regulation deserve comment. First, the proper denominator for receptor concentration is uncertain. It is not clear whether cellular sensitivity is most clearly related to the number of receptors per cell, per square micrometer, per receptor cluster, per milligram membrane protein, or per unit adenylate cyclase and 5'-nucleotidase activity. This becomes an important issue when a cell undergoes significant hypertrophy, as the adipocyte does in obese animals. In such cases, therefore, it is important to measure as many aspects of cell membrane function as possible. At present, hormonal sensitivity seems to correlate best with the number of receptors per unit surface area. A second major problem is technical and arises from cellular heterogeneity and possible difference in selection of cell or membrane populations when studying tissues or cells in different states. These considerations stress the need for a careful characterization of the tissue preparation being studied

(see reference 105 for a detailed discussion). Finally, it is worth noting that alterations in receptor affinity may also occur and play a role in altering cellular sensitivity to hormone (77). At present, no studies have clearly demonstrated synthesis of a mutant or abnormal receptor protein.

#### VIII. COUPLING OF HORMONE RECEPTORS TO HORMONE EFFECTORS

There have been a number of attempts to quantitatively correlate the binding of a hormone to its receptor and the biological effects of the hormone. In most of these, the hormone receptor-effector complex has been treated as a single molecule (Fig. 1, left), and the modeling has followed a simple receptor occupancy theory (18). In this case, the response of a target tissue to a hormone is considered directly proportional to the number of receptor sites filled, i.e.,  $\text{Effect} = \alpha [HR]$ . This implies that a maximal response is obtained only when all receptors are occupied and that the binding and biological response curves are superimposable over their entire range. Such a close quantitative correlation is rarely observed. Three deviations from the occupancy model have been frequently noted.

First, in a variety of systems a maximum biological response is achieved with only a minority of receptors occupied. For example, maximal stimulation of glucose oxidation by isolated adipocytes occurs when only 2-3% of insulin receptors are occupied (bottom of Fig. 8) (82, 97); similarly, maximal stimulation of steroidogenesis by Leydig cells occurs when only 1% of LH-hCG receptors are occupied (15). The additional 97-99% of receptors have been termed "spare" or "reserve" receptors. These should not be considered with inactive receptors. Other biological responses in these or other cells may require greater levels of receptor occupancy (83) (top of Fig. 8). Furthermore, at present, there are no data to suggest that the 1-3% of receptors necessary for these biological responses differ from the remainder.

A second type of deviation from the occupancy model has been observed in which there are no spare receptors, but the biological effect is not a simple linear function of receptor occupancy. For example, adenylate cyclase activation by lysine-vasopressin is 80% of maximum at only 10% receptor occupancy, although complete saturation of the receptor sites is required to obtain maximum activation (12, 70). Furthermore, the bind-



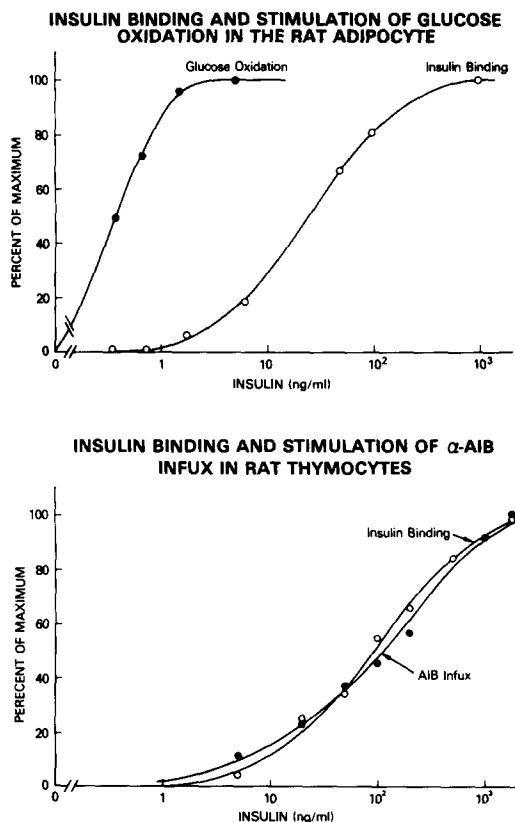


FIGURE 8 Correlation between insulin binding and biological activity in two systems. (Top) Insulin binding to isolated adipocytes and insulin-stimulated glucose oxidation in these same cells are plotted as a function of insulin concentration. Note that glucose oxidation is maximal when about 5% of receptors are occupied. C. R. Kahn and K. Baird, unpublished observations. (Bottom) Insulin binding to isolated thymocytes and insulin-stimulated  $\alpha$ -aminoisobutyric acid transport (AIB) in these same cells are plotted as a function of insulin concentration. Note the close correlation between insulin binding and stimulation of amino acid transport. (Replotted from the data of Goldfine et al. [58].)

ing of lysine-vasopressin is positively cooperative (Hill coefficient = 1.42), while the activation of the adenylate cyclase by lysine-vasopressin is negatively cooperative (Hill coefficient = 0.35). The degree of nonlinearity is variable depending on the vasopressin or oxytocin analogue studied (70). A similar type of nonlinear coupling has been found for glucagon binding and cyclic AMP (cAMP) generation in isolated hepatocytes (129). Despite the spare receptors and nonlinear coupling, Gliemann et al. (54) and Bockaert et al. (12) have found close correlations between the

time course of receptor occupancy and the onset of biological effects with insulin and vasopressin.

Finally, deviations from the simple occupancy model can occur due to differences in "intrinsic activity" of the hormone analogues; different analogues may have varying capacity to initiate a biological response after occupying the receptor. In the most extreme case, a hormone analogue may produce no biological response upon occupying the receptor; this is the case of the true competitive antagonist. Compounds with a low efficacy in inducing a biological response possess properties intermediate between agonists and antagonists and have been termed "partial agonists." While examples of antagonists and partial agonists are plentiful in the studies of neurotransmitters (2, 14, 103, 119), in most cases, decreased biological potency of a peptide hormone analogue is due to a decreased affinity for the receptor rather than an alteration in "intrinsic activity" (see Fig. 3) (39, 44, 53, 70).

The failure to observe a close correlation between hormone action and direct measures of the hormone binding to its receptor is not surprising in view of the constraints imposed by the simple model of a single molecule which possesses both receptor and effector functions (Fig. 1, left). Clearly, the model must be more complex to account for already known cooperative interactions among receptors (26-29, 35, 70, 92, 94) and regulatory effects of guanyl nucleotides, Mg, and other heterotropic effects on hormone binding or adenylate cyclase (10, 55, 128, 142). In addition, a model which employs separate mobile molecules for the receptor and effector functions which preferentially interact when the receptor is occupied by hormone would help to explain spare receptors, nonlinear coupling, and possible differences in intrinsic activity. Such a model is discussed in more detail below.

## IX. RELATIONSHIP OF HORMONE RECEPTORS TO A FLUID MOSAIC MEMBRANE

In 1972 Singer and Nicolson (138) introduced the fluid mosaic membrane model. In this model, the membrane is pictured as a two-dimensional solution of globular integral proteins dispersed in a relatively fluid lipid bilayer. Some of these proteins extend completely through the bilayer, while others are partially embedded in or on the surface of the membrane. Further, some membrane components appear relatively immobile, while others

are capable of rapid lateral mobility. Most hormone receptors appear to be integral membrane proteins, i.e. they require strong detergents capable of breaking hydrophobic bonds for dissociation from the membrane and are usually insoluble in neutral aqueous buffers. At present, it is not known whether these receptors span the entire membrane or are partially embedded in the membrane, whether they are relatively mobile or immobile, or whether they are monomeric or oligomeric.

The concept that membrane fluidity may play an important role in hormonal activation of cellular processes was also originally proposed by Singer and Nicolson (138) and has since been amplified by Perkins (113), Cuatrecasas (22), de Häen (25), and others (26-29, 92). Coupled with possible ligand-induced changes in receptor conformation, this may offer a more complex but complete picture of hormone membrane interaction.

In these models, the hormone receptor (*R*) and effector (*E*) are depicted as separate integral membrane components which are capable of diffusing laterally in the membrane plane. Hormone activation of a cell is then the result of at least two sequential steps:



which occurs on the surface of the membrane and



which occurs within the membrane. Such a model is depicted in a highly schematic fashion in Fig. 9. In some cases, a third intermediate component has been postulated which couples the *HR* complex to the effector (Fig. 1, right). This general model provides a means for explaining spare receptors and nonlinear coupling of receptors and effectors and would present a possible mechanism by which multiple hormones could affect a single effector system and a mechanism by which a single hormone could influence multiple effector systems.

In the simplest case, the membrane contains a 1:1 stoichiometric relationship between receptors and effectors (Receptor 2 in Fig. 9) and the  $\overline{HR}$  complex is highly mobile and has a high affinity for the effector. In this case, every receptor occupied by hormone would be rapidly coupled to effector, and the biological response would be a direct function of the percentage of the total receptor population occupied by hormone. Alternatively, receptors and effectors may not be present in stoichiometric amounts (25, 113) (Receptor 1 in Fig. 9.). Spare receptors would simply be the result of an excess of receptors with respect to effectors of the given biological response. If a single class of receptors

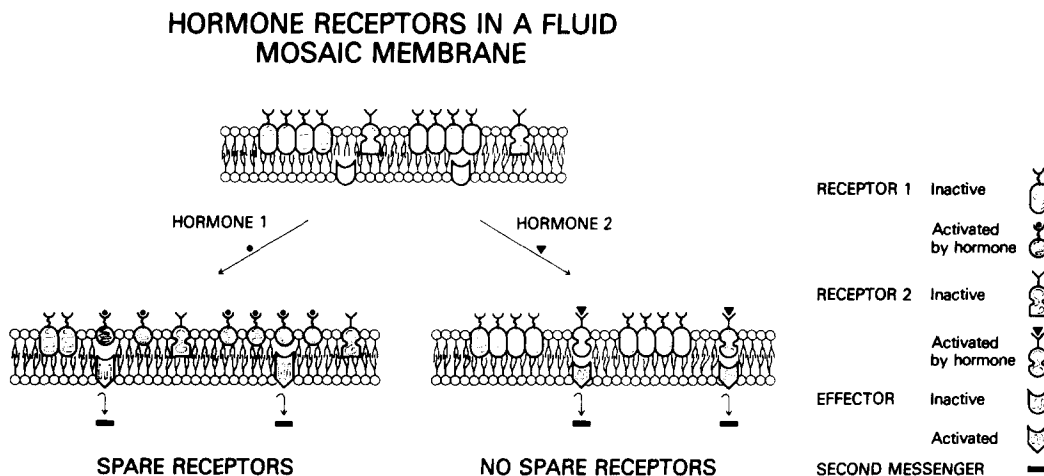


FIGURE 9 A highly schematic model of hormone receptors in a fluid mosaic membrane. Receptors for two hormones are depicted. Receptor 1 exists in an oligomeric state and depolymerizes upon binding of the hormone. The free receptor subunits then migrate and interact with effectors. Since there are four receptors per effector, there would appear to be spare receptors. Receptor 2 exists as a monomer and in a one-to-one ratio with the effector; there are no spare receptors in this case. This is a highly schematic representation and numerous other possibilities exist, including polymerization or clustering of receptors upon hormone binding. (Modified after references 22, 25, 113, 138.)

interacts with more than one type of effector, the number of spare receptors may differ for the two systems. For example, only about 3% of insulin receptors need to be occupied to produce maximal glucose oxidation in adipocytes (43, 82), while nearly 100% need to be occupied to produce maximal amino acid transport in thymocytes (58) (Fig. 8). In the first case there appear to be 30 receptors for every effector, while in the second the ratio appears to be 1:1 or more.

Different types of receptors and effectors may also be capable of interacting. A single adenylate cyclase, for example, might be stimulated by more than one hormone, although each hormone has its initial interaction with a separate receptor. This would explain the ability of multiple hormones to activate liver and adipose adenylate cyclase and would explain why the activation is not additive at maximal hormone concentrations (125). Such a model would also explain how a hormone might produce multiple biological responses by interaction of its receptor with several types of effectors. In liver, for example, catecholamine effects on glycogenolysis appear to be mediated via cAMP while those on gluconeogenesis do not (148).

The nature of the coupling between hormone binding to receptors and biological effects will depend on the relative mobility of the two components in the membrane, the probability of a collision between the receptor and effector, other interacting membrane components, and the nature of the final linkage between the hormone-receptor complex and the effector. The hormone may influence the degree of aggregation among receptors, the affinity of the receptor for the effector, the relative mobility of the receptor in the membrane plane, or the ability of the receptor to activate the effector. In some cases, it is possible that the hormone induces aggregation or dissociation of receptor subunits. Such models could account for the combination of negative cooperativity in binding and positive cooperativity in biological response<sup>7</sup> and could provide a mechanism by which an oligomeric cluster of noncooperative receptors could generate a cooperative biological response (93). Changes in hormone structure could result in changes in the properties of the hormone-receptor complex and account for the agonist-antagonist properties of the molecule. Other factors such as ions, guanyl nucleotides, and changes in membrane lipids might also influence hormone action

<sup>7</sup> De Meyts, P. Personal communication.

by altering any of the factors involved in the chain of events which comprise the functional linkage between the receptors and effectors. At present, direct demonstration of the migration of receptors for peptide hormones or neurotransmitters has not been accomplished. Several bits of data suggest that this type of model may be correct.

The most direct examples of the possible importance of a fluid mosaic membrane with respect to the receptors is the demonstration of site-site interactions among some receptors (see section V, 3). While these may be due to conformational changes in multisubunit protein receptors, more data suggest that the polymerization or depolymerization of clusters of receptors may be important. Electron microscope studies of ferritin-insulin binding to fat cells and liver membranes have demonstrated clustering of receptors (Fig. 5) (71, 110). In addition, De Meyts et al. have found that, when studied as a function of temperature, negative cooperativity shows a sharp inflection point in an Arrhenius plot at about 21°C (27). A sharp transition at about this temperature has also been associated with formation of clusters in artificial lipid bilayers (86) or a phase transition of membrane phospholipids (135). In addition, concanavalin A which inhibits migration of a variety of receptors and surface antigens also inhibits the site-site interactions among insulin receptors (26). Insulin receptors appear to retain their cooperative properties when solubilized, suggesting that complete membrane integrity is not required for the site-site interactions (51). Interestingly, the soluble insulin receptors appear to undergo a change in size when cooperativity is induced (51).

TSH binding also appears to be related to membrane fluidity with evidence for both negativity<sup>4</sup> (146, 151) and a rather sharp increase in the number of TSH receptors at about 30°C (3). Using membrane-bound fluorescent probes, Bachford et al. found a similar temperature dependence of fluorescence polarization, suggesting an increase in thyroïdal membrane lipid fluidity at this temperature (3). Like insulin receptors, TSH receptors appear to retain some of their cooperative properties when removed from the membrane by detergents (146). A relationship between ACTH binding and fluidity of membranes from adrenal cortical cells has also been found; in this case, a temperature transition occurred at 23°C (3). In a similar line, Craig and Cuatrecasas have demonstrated temperature-dependent redistribution of cholera toxin receptors (20). An abrupt

increase in glucagon- and epinephrine-stimulated hepatic adenylate cyclase at temperatures above 32°C suggestive of a cooperative change, has also been observed (84), but at present this has not been correlated with any change in hormone binding.

More generalized changes in membrane structure in response to hormones have been suggested by spectrofluorometric studies. Exposure of appropriate tissues to either human growth hormone (131) or TRH (69) has been found to result in a quenching of intrinsic tryptophan fluorescence. In both cases, the change results from occupation of only a small number of sites, and inactive analogues do not produce the effect. Storm and Chase (144) also found evidence for a glucagon-induced change in the membrane as measured by enhanced labeling of a 240,000 mol wt component of plasma membrane by iodoacetic acid or iodoacetamide in the presence of glucagon. The kinetics of labeling correlate well with the kinetics of inactivation of adenylate cyclase by iodoacetamide, suggesting that the hormone induced a conformational change in the effector system (adenylate cyclase) which resulted in selective binding of some component of this system.

#### X. SUMMARY

Receptors for peptide hormones and neurotransmitters are integral components of the plasma membrane of cells which serve to couple the external milieu to the intracellular regulators of metabolism. These macromolecules are usually high molecular weight glycoproteins, and in many cases appear to have more than one subunit capable of binding the hormone. The interaction of the hormone or neurotransmitter with its receptor is rapid, reversible, and of high affinity and specificity. Many receptors exhibit cooperative properties in hormone binding or biological function. The concentration of receptors on the membrane is a function of continued synthesis and degradation, and may be altered by a variety of factors including the hormone itself. The fluid mosaic nature of the membrane may allow hormone receptors and effectors to exist in free floating states. Further investigations of the hormone-receptor interaction will no doubt yield new insights into both the mechanism of hormone action and membrane structure and function.

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