RECEPTORS IN PHARMACOLOGY

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PREFACE

The study of receptors today constitutes one of the most rapidly expanding areas of cellular biochemistry and molecular biology. An array of new techniques is being developed to study the kinetics, structure, and mode of operation of these key elements in the control of cellular function, in particular in vitro techniques for the study of the isolated, solubilized receptor. This book presents up-to-date surveys of the most promising areas in this field including receptor theory, receptors for acetylcholine, progesterone, amino acids, insulin, the β-adrenergic receptors, opiates, the sialoglycoprotein of erythrocyte membranes, the coupling of hormone receptors to adenylcyclase, and biophysical aspects of ion flux in excitable membranes.

This work is of importance not only in the area of basic science but also for the practice of medicine. Not only do the hormones widely used in medicine exert their most significant action at these receptors, but many drugs in general use also operate as agonists and antagonists at these same receptors. Thus these new developments are of relevance and importance to all biochemists, pharmacologists, physicians (in particular endocrinologists), cell biologists, and molecular biologists.

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Chapter 1

RECEPTOR THEORY

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DRUG-RECEPTOR INTERACTIONS

The concept that chemical agents which initiate or modify cellular response might do so through action at specific cellular locations was first advanced by J. N. Langley in 1878 [1] as a result of his investigations into the mutually antagonistic actions of atropine and pilocarpine on cat salivary flow. Langley wrote, "We may, I think, without much rashness, assume that there is some substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors." Although it was Paul Ehrlich who actually introduced the term "receptor," viewing it as a "combining group of the protoplasmic molecule to which the introduced group is anchored," it was Langley who utilized the term in the context that now appears most useful. As a result of his investigations of the antagonistic effects of curare on nicotine stimulation of skeletal muscle Langley concluded [2], "Since neither curare nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curare is not identical with the substance which contracts. It is convenient to have a term for the specially excitable constituent, and I have called it the receptive substance. It receives the stimulus, and by transmitting it, causes contraction." Langley further concluded, "Since the formation of the nicotine compound causes contraction, and that of the curare compound does not, it is obvious that the chemical rearrangements set up in the muscle molecule by the combination of one of its radicals are different in the two cases. In fact, it seems probable that a special radical is necessary for the combination with a number of chemical bodies, and that the compound formed leads to further change depending upon the nature of the compound."

Langley thus drew attention to two fundamental characteristics of the receptor, namely, a recognition capacity for specific ligands or classes of ligands and an ability, as the ligand-receptor complex, to initiate a biological response. These characteristics, the specific binding of the ligand and the relationship between binding and response, will form the principal focus of discussion in this chapter. The discussion will be exclusively concerned with receptors which are integral components of the cell membrane and will focus largely, but not exclusively, on receptors for neurotransmitters.

A. The Classification and Organization of Receptors

Receptors may be classified by the response that they initiate, contraction, hyperpolarization, secretion, etc., but it is immediately obvious

TABLE 1

Ionic Processes Mediated by Acetylcholine [3]

Site	Membrane potential change	Ion conductance change	Ref.
Vertebrate skeletal muscle	Depolarization	$Na^{+\uparrow}$, $K^{+\uparrow}$	4
Sympathetic ganglia	Depolarization	$Na^{+\uparrow}$, $K^{+\uparrow}$	5
Vertebrate heart	Hyperpolarization	$K^{+\uparrow}$	6
Cat cerebral cortex	Depolarization	$K^{+\downarrow}$	7
Snail neurons	Depolarization	$Na^{+\uparrow}$	8,9
	Hyperpolarization	$Cl^{-\uparrow}$	
Aplysia neurons	Depolarization	$Na^{+\uparrow}$	10,11
	Hyperpolarization (fast)	$Cl^{-\uparrow}$	
	Hyperpolarization (slow)	$K^{+\uparrow}$	

that this is not very satisfactory. Thus, the fat cell responds with lipolysis to a number of different hormones including epinephrine, glucagon, thyroid stimulating hormone, luteinizing hormone, growth hormone, adrenocorticotrophic hormone and secretin and to simply refer to the "lipolysis receptor" totally obscures the large differences in recognition specificity that are involved even though all agents promote lipolysis through the same process, activation of adenylate cyclase. Similarly, where the responses are quite different the recognition specificity may be identical as, for example, with acetylcholine action on the intestine and heart where excitatory and inhibitory responses, respectively, are both mediated through an atropine-sensitive acetylcholine receptor. Thus, an identical recognition mechanism may be linked to different response mechanisms as can be seen clearly in Table 1 showing the nature of the ion channels affected by acetylcholine in a variety of preparations. Such findings suggest that the receptors are probably best regarded as composed of at least two components -- a recognition component determining ligand specificity and an associated catalytic or amplification component mediating the response. These two components may represent different sites on the same macromolecule or on quite different macromolecules but either possibility will

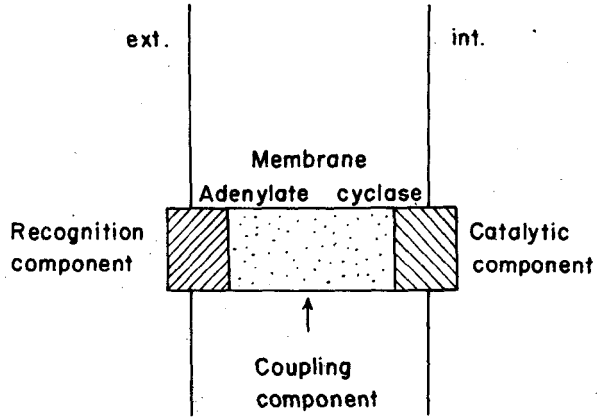

FIG. 1. Schematic representation of adenylate cyclase showing the external recognition component and the internal catalytic component linked by a transmembrane coupling component.

require a linkage process to couple the recognition component to the amplification component. Evidence to confirm or distinguish between these possibilities is not generally available, but this type of analysis serves to emphasize the clear analogy between receptors and regulatory enzymes where ligand binding to a site distinct from the catalytic site serves to modify activity at the latter [12-14].

The multicomponent receptor model has been widely used for the hormonally sensitive membranal adenylate cyclase systems in which an externally located recognition site is coupled to an internally located catalytic site (Fig. 1) [15]. A number of lines of evidence support this model. Thus, fluoride ion, believed to activate directly at the catalytic site, does not activate intact cells but does activate membrane fragments or inverted cells [16-18]. Additionally, proteolytic enzymes can readily destroy hormonal sensitivity without affecting fluoride sensitivity [19-21]. Similarly, the receptors mediating chemotaxis in bacteria also appear to have at least two components of which a binding protein is necessary but not sufficient, since although mutants lacking this protein do not show chemotaxis, mutants containing the protein may also fail to show the chemotactic response [22,23]. A similar conclusion may be permissible for Na^+, K^+-ATPase which consists of a large and a small polypeptide chain with the small component possibly functioning as the Na^+ ionophore [24,25]. Recent developments in the isolation and purification of the acetylcholine receptor glycoprotein from Electrophorus electricus suggest that a similar two component model is applicable here also [26]. The complex isolated appears to consist of 4-6 subunits which may be nonidentical since there

are twice as many binding sites for the antagonist α-bungarotoxin as there are for acetylcholine and, furthermore, when this protein is reconstituted into phospholipid vesicles, some ion translocation occurs in response to cholinergic agonists. It is possible that one type of subunit represents the recognition component and the other an ion channel component.

The receptors that will be discussed in this chapter are membrane components. Evidence that localizes such receptors to the cell membrane has been derived through a number of experimental techniques. Thus, acetylcholine only exerts its depolarizing action at the skeletal neuromuscular junction when applied to the surface and is inactive if injected intracellularly [27]. A number of agents have been covalently attached to nonpenetrating Sepharose or glass beads and essentially full biological activity is retained [28-31]. Ligand binding has been observed in many instances with cell membrane fragments which also retain physiological responsiveness [32]. Finally, ligand binding at the cell membrane surface can be directly observed and quantitated as in the case of the acetylcholine receptor through autoradiographic localization of [^{3}H]α-bungarotoxin, a specific receptor antagonist [33,34]. In the latter case the distribution of binding corresponds to the distribution of the 80-120 Å particles observed in freeze-fractured synaptic membranes [35].

Since receptors are part of the cell membrane and function in the environment of the membrane it is to be anticipated that their properties and mechanisms of action will be dependent upon this membrane environment. It is currently accepted that the membrane is best regarded as a fluid phospholipid bilayer into which are incorporated peripheral and integral proteins (Fig. 2) [36-38] making up the major constituents of functional membranes. The membrane receptors are best regarded as integral proteins that are incorporated into the phospholipid bilayer and which require vigorous treatment (detergents etc.) to be dissociated from the membrane. It is known that the activities of many membrane-bound enzymes are influenced by lipids, sometimes with considerable specificity [39,40], and it is to be anticipated that receptors will be no less influenced by phospholipid function. Indeed, solubilized adenylate cyclase has been reported to require phosphatidylserine for glucagon activation [41] and phosphatidylinositol for norepinephrine activation [42].

A basic feature of the fluid membrane model is the mobility of the membrane constituents, represented particularly by lateral diffusion in the membrane plane. There is much evidence to support the concept that both phospholipids and proteins do exhibit lateral diffusion with diffusion constants in the range 10^{-8}-10^{-9} cm^2 sec^{-1} [43]. However, not all membrane components are so mobile and there are obviously membrane areas where substantial structural homogeneities exist and are maintained. This is true for the organization of synaptic membranes, particularly in skeletal neuromuscular junctions, where the subsynaptic membrane may consist

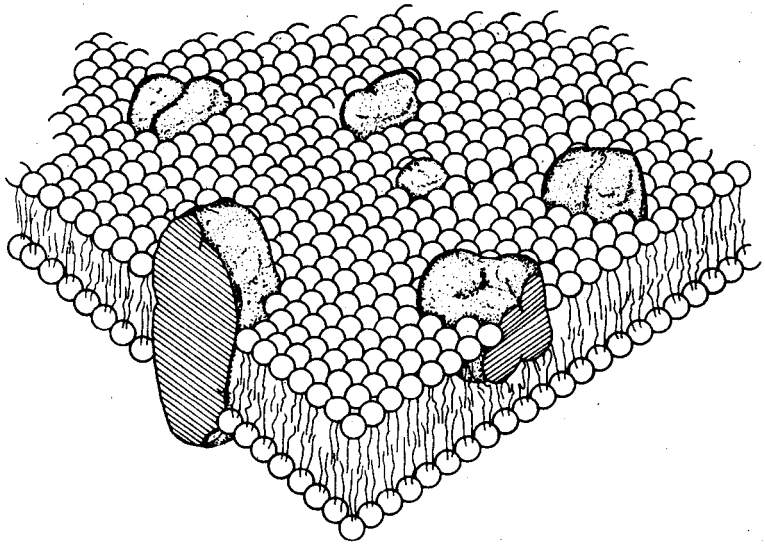

FIG. 2. Membrane organization according to the fluid mosaic model. The solid bodies represent proteins that may be randomly distributed. (Reproduced with permission from Singer and Nicolson [38].)

largely of receptor protein ($\sim 30,000$ receptors/μm^2) [34] and where the presynaptic membrane is also highly specialized for the release of neurotransmitter [44].

The concept of membrane fluidity may have important consequences for receptor organization. Thus, Cuatrecasas [32] has suggested that the recognition (R) and amplification (A) components of receptors may not be normally physically associated and that association is initiated through the formation of the hormone-R complex (Fig. 3). This scheme may be attractive for systems such as adenylate cyclase, which in a single cell may be multiply and specifically sensitive to a number of hormones, since it avoids the requirement for all of the surface located recognition sites to be in contact with a single active site. This model does not require a 1:1 stoichiometry of recognition and amplification components and it is quite plausible that there may exist an excess of recognition sites or that a single laterally diffusing hormone-recognition site complex might sequentially interact with several different amplification components thus providing a basis for the modulation of separate and distinct membrane functions by a single ligand. It is unlikely, however, that this model is applicable to all receptor systems such as the extremely rapidly responding acetylcholine receptors but it is an attractive possibility for the more slowly responding multiregulatory adenylate cyclase systems.

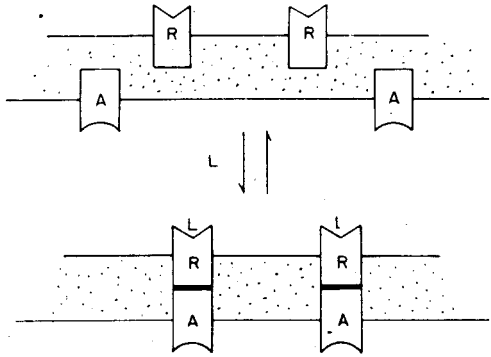

FIG. 3. Representation of "floating" receptor model in which the recognition (R) and amplification (A) components are unassociated but become linked and functional when ligand binds (After Cuatrecasas [32].)

The preceding analysis suggests some of the problems that are encountered in the attempted quantitative description of ligand-receptor interactions. The basic problem is that of describing a sequence of events of unknown complexity,

$$A + R \rightleftharpoons AR \xrightarrow[\text{steps}]{\text{intermediate}} \text{Response} \qquad (1)$$

in which the observed response may be several stages removed from the primary ligand binding step. Furthermore, all of the events of Eq. (1) are likely to be considerably influenced by the membrane environment and upon removal from this environment not only may the response be lost but the basic kinetic features of the interaction may be significantly changed. In addition to these intrinsic complexities there will be a number of experimental complexities to be dealt with including, in the case of tissue work, problems of heterogeneity of cell type, diffusion barriers, ligand interactions at sites other than that being studied, various mechanisms of ligand removal and/or destruction all of which may grossly perturb the relationship between ligand concentration and response [45].

The attempted formulation of quantitative treatments of drug-receptor interactions is clearly a formidable problem. Since receptors are defined both by their binding specificity and by the responses that they initiate it seems clear that the definition of the relationship between binding and response must be a prime objective in any quantitative analysis of receptor mediated events.

TABLE 2

Reaction Rates of Ligand-Antibody Reactions [52]

Antibody	Digoxin k_1[a]	Digoxin k_{-1}[b]	Ligand ouabain k_1	Ligand ouabain k_{-1}	Digitoxin k_1	Digitoxin k_{-1}
Digoxin	0.93	1.9	1.5	170	1.4	7.2
Ouabain	1.3	64	0.87	15	1.4	38
Digitoxin	1.1	12	1.3	140	1.4	2.3

[a] $\times 10^7$ M^{-1} sec^{-1}.

[b] $\times 10^{-4}$ sec^{-1}.

B. The Elementary Rates of Drug-Receptor Interactions

The advent of fast reaction techniques has allowed the measurement of the rates of association of many small ligands with proteins [Hammes, 55]. In general the reaction between a ligand protein,

$$L + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LP \qquad (2)$$

is very fast with k_1 approaching the maximum value, 10^7-10^9 M^{-1} sec^{-1}, calculated for diffusion-controlled reactions [46]. Since these reactions are generally specific this means that the affinity of the ligands should be determined by k_{-1}. Recent investigations of ligand-antibody reactions lend support to this proposal (Table 2) since the values of k_1 are very similar but k_{-1} varies over two orders of magnitude. Unfortunately, there are very few measurements of the kinetics of drug-receptor interactions: values for the insulin-receptor, oxytocin-receptor, glucagon-receptor, cobratoxin-acetylcholine receptor, and strychnine-glycine receptor of 8.5×10^6, 7.2×10^5, 1.6×10^6, 10^5, and 10^7 M^{-1} sec^{-1} have been reported [47-51] and these are close to, but somewhat less than, the diffusion controlled limit.

The importance of the values of k_1 and k_{-1} lie in the deductions that can be made concerning the parameters controlling the chemical specificity of the drug-receptor interaction. Although the stability of ligand-protein

complexes is probably generally determined by k_{-1} there is evidence that this may not always be so. Thus, in the interaction of sulfonamides with carbonic anhydrase the affinity of the ligands is controlled largely by k_1 which shows greater variation with ligand structure than does k_{-1} [53, 54]. It is probable that formation of the sulfonamide carbonic anhydrase complex involves a multistep process with an initial rapid formation of a preequilibrium complex.

Rather, generally ligand-protein interactions have been shown to involve a two step pathway,

$$L + P \rightleftharpoons LP \longrightarrow LP^* \qquad (3)$$

with the time constant for the second step being typically 10^{-2}-10^{-4} sec [55] and probably being associated with the catalytic process. The rate processes thus far discussed are all very fast, but proteins can also undergo ligand-induced changes that are extremely slow. Frieden [56] has referred to enzymes which respond slowly to a rapid change in ligand concentration as hysteretic enzymes and has indicated that slow conformational changes, polymerization/depolymerization and displacement of tightly bound ligand may be determinant for such changes. There are many examples of such hysteretic enzymes including the activation of yeast glyceraldehyde-3-phosphate dehydrogenase by NAD (time scale seconds), D-amino acid oxidase by FADN (time scale minutes) and isoleucine inhibition of threonine deaminase (time scale minutes). At the receptor level such phenomena have not yet been well investigated; however, the interaction of neurohypophyseal hormones with bovine renal medullary adenylate cyclase shows an initial burst of cAMP production lasting for 2-4 min followed by a linear rate suggesting a slow hormone-induced change of the enzyme state [57]. It seems probable also that the process of receptor desensitization induced by activator ligands which has a time scale of seconds or minutes may also represent an example of a slow conformational change.

Attempts to define the elementary kinetics of the various steps that may be involved in receptor activation processes are fraught with obvious difficulties not least of which is the unknown complexity of the steps involved in the activation process. Furthermore, experiments with bath application to isolated tissues, although simple to perform, suffer from the disadvantage that diffusion of the drug to and from the receptors may constitute the rate-determining steps. Even if this is not so it may be extremely difficult to distinguish between diffusion limiting and receptor limiting models of drug action. However, some progress is being made in the kinetic analysis of the action of acetylcholine at the skeletal neuromuscular junction. At this receptor system the sequence of events is believed to be (i) diffusion of acetylcholine to the receptors; (ii) combination of acetylcholine with receptors; (iii) response of receptor mechanism giving rise to conductance change (opening and closing of ion channels); (iv) removal of acetylcholine. The release of one vesicle (quantum) of acetylcholine gives rise to a miniature endplate current (mepc) which has a rise