Neurotransmitter Receptor Binding

Second Edition

Editors

Henry I. Yamamura, Ph.D.
Departments of Pharmacology, Biochemistry, and Psychiatry
College of Medicine
The University of Arizona
h Sciences Center
cson, Arizona

S. J. Enna, Ph.D.
Departments of Pharmacology, Neurobiology, and Anatomy
The University of Texas Medical School at Houston
Houston, Texas

Michael J. Kuhar, Ph.D.

Neurotransmitter Receptor Binding

Second Edition

Editors

Henry I. Yamamura, Ph.D.

Departments of Pharmacology, Biochemistry, and Psychiatry
College of Medicine
The University of Arizona
Health Sciences Center
Tucson, Arizona

S. J. Enna, Ph.D.

Departments of Pharmacology, Neurobiology, and Anatomy
The University of Texas Medical School at Houston
Houston, Texas

Michael J. Kuhar, Ph.D.

Departments of Neuroscience, Pharmacology, and Psychiatry
The Johns Hopkins University School of Medicine
Baltimore, Maryland

Raven Press ■ New York

Raven Press, 1140 Avenue of the Americas, New York, New York 10036

Made in the United States of America

Library of Congress Cataloging in Publication Data
Main entry under title:

Neurotransmitter receptor binding.

Includes bibliographies and index.
1. Neurotransmitters—Analysis. 2. Neurotransmitter receptors. 3. Radioligand assay. I. Yamamura, Henry I. II. Enna, S. J. III. Kuhar, Michael J. [DNLM: 1. Binding Sites. 2. Neuroregulators. 3. Radioligand Assay. 4. Synaptic Receptors. QV 126 N494]
QP364.7.N47 1985 599'.0188 84-17876
ISBN 0-88167-027-8

Preface to First Edition

The use of ligand-binding techniques to study neurotransmitter receptor sites has led to a broad range of important biological advances. It is now possible to obtain information about the regional distribution of neurotransmitter receptors in brain, the pharmacological, biochemical, and developmental characteristics of these sites, and the functional interrelationships between neuronal cell types. In addition, the receptor-binding procedure has been used as an assay technique to measure levels of neurotransmitters and drugs, to identify new transmitter candidates, and to separate and purify receptors. The simplicity and versatility of these techniques render them attractive biochemical tools for routine use in neuroscience laboratories.

As with all methods, it is necessary to understand some of the basic principles of the technique in order to use a receptor-binding procedure appropriately. To this end, this text covers the fundamental principles of neurotransmitter receptor-assays, discusses the precise methodology for some of the more commonly used assays, and describes ways in which the assays can be used in neurobiological investigations, drug screening, neurochemical and histochemical analysis, and in the solubilization and isolation of receptors. This book is not intended as a review of the literature in these areas, but rather as a guide to enable neuroscientists, from technicians to senior investigators, to understand the basis of receptor-binding assays and to help establish these procedures in the laboratory. The utility and limitations of the binding assays in industrial drug screening, clinical medicine, and basic research are stressed.

Our chief goal has been to provide the reader with fundamental principles and procedures. Discussions of specific studies are limited to those that illustrate and clarify these principles. By using this approach, we hope that this book will serve not only as a laboratory guide but also as a catalyst for future investigations.

Henry I. Yamamura
S. J. Enna
Michael J. Kuhar

Preface to Second Edition

It has been over six years since we edited the first edition of *Neurotransmitter Receptor Binding*. Although the basic criteria and principles of radioligand-receptor binding has not changed over this period, many refinements and vast improvements have been made in the technical aspects of ligand-receptor binding. All of the chapters have been revised and we have included new chapters on neuropeptide and voltage-sensitive ion channel binding methods.

We have made a conscious effort not to cite the enormous literature that has been compiled in this field in the past decade, rather our intent and goal has been to provide the investigator with fundamental principles and procedures consistent with the past edition.

This revised laboratory guide will be useful to everyone in the field.

Henry I. Yamamura
S. J. Enna
Michael J. Kuhar

Contributors

James P. Bennett, Jr. *Department of Neurology, University of Virginia, School of Medicine, Charlottesville, Virginia 22904*

David R. Burt *Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201*

William A. Catterall *Department of Pharmacology, University of Washington, School of Medicine, Seattle, Washington 98195*

Ian Creese *Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, California 92093*

S. J. Enna *Departments of Pharmacology and of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025*

Michael Hanley *Department of Biochemistry, Imperial College, London SW7 2AZ, England*

Morley D. Hollenberg *Endocrine Research Group, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1*

Michael J. Kuhar *Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Jon Lindstrom *The Salk Institute, San Diego, California 92138*

Solomon H. Snyder *Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Henry I. Yamamura *Departments of Pharmacology, Biochemistry, and Psychiatry, University of Arizona, College of Medicine, Tucson, Arizona 85724*

Introduction

New Themes in Neurotransmitter Receptor Binding

Neurotransmitter receptors consist of two components, the recognition or binding site and the "translation" component which converts the recognition information into altered cellular function. During the last 10 years, some dramatic and rapid developments in neurotransmitter research have dealt with binding sites. In addition to the importance of receptor binding as a means of exploring molecular aspects of synaptic transmission at the most fundamental levels, binding studies have proven to be of immense practical importance as rapid and simple screening techniques in the pharmaceutical industry for developing new therapeutic agents and as tools for measuring drug levels in blood and tissues by radioreceptor assays.

One goal of receptor research is to purify and isolate receptor proteins to elucidate the molecular mechanisms whereby neurotransmitter recognition alters ion permeability. The greatest success in this area has come with the nicotinic cholinergic receptor in the electric organ of various invertebrates. Several laboratories have isolated the protein subunits of the receptor. Classic chemical techniques have elucidated the amino acid sequence of some of these subunits. Isolated receptors have been reconstituted into artificial lipid membranes capable of mediating sodium flux in a fashion that is stimulated selectively by acetylcholine. This work establishes definitively that a single protein molecule contains both the recognition site for acetylcholine and the sodium ion channel.

Purification of β-adrenergic receptors also has advanced considerably. Several laboratories have isolated homogeneous β-receptors and raised antisera against them. A major question remains as to how catecholamine recognition triggers an interaction between the receptor, the guanosine 5′-triphosphate (GTP)-binding "N"-protein, and adenylate cyclase. Theoretical models indicate changes in the affinity of β-receptors for catecholamines when the receptor binds to the N-protein. Ways in which these interactions influence GTPase activity and the subsequent activation of adenylate cyclase have been characterized. Perturbations of this macromolecular complex during the process of catecholamine-induced desensitization also have been elucidated.

Very recently, recombinant DNA techniques have been applied to existing research efforts, especially in the case of the nicotinic cholinergic receptor. Several groups have cloned genes for certain subunits of the receptor. Genes for all four subunits of the receptor have been cloned, and a three-dimensional model of the receptor and its sodium channel has been developed. This very detailed molecular information comes close to answering the critical question of how acetylcholine recognition at one site on the protein alters sodium passage through another portion of the same molecule.

Characterizing multiple subtypes of neurotransmitter receptors is another developing theme. In the case of the opiate receptor, at least three distinct receptor-binding sites can be designated: μ, δ, and κ. These binding sites have their physiological correlates in differential actions of drugs on various types of smooth muscle preparation. Two subtypes of benzodiazepine receptors, referred to as type I and II, have been differentiated. It is generally believed that at least three forms of muscarinic cholinergic receptors can be distinguished, both on binding and on functional grounds. Researchers differentiate among three or four subtypes of postsynaptic dopamine receptors, not including autoreceptors.

The subtle distinctions among the numerous receptor subtypes often seem forbidding to those who are not aficionados of the field. Some of the uninitiated may wonder if the focus on complex subtypes of the receptors with esoteric nomenclature reflects less science than a clannish effort to turn away "outsiders." Although much work in the multiple-receptor field may seem confusing at this early stage of development, the theoretical and practical consequences of this work are considerable. Exquisitely subtle information processing may result if, for instance, different forms of a neuropeptide interact differentially with various receptor subtypes. In drug development, agents that may be selective for one or another subtype of a receptor may offer greater therapeutic selectivity with less severe side effects.

A further subtlety of receptor-binding studies relates to the appreciation of receptor complexes. The β-receptor–N-protein–adenylate cyclase complex has been recognized for several years. Similar subunit interactions occur in dopamine receptor complexes. Distinct components of a γ-aminobutyric acid (GABA) receptor complex may differentially mediate the actions of various drugs. Thus, benzodiazepines act at specific receptors that are unique proteins separate from the GABA receptor protein to which they are linked allosterically. Barbiturates and convulsants, such as picrotoxinin, act at yet another site that is associated with the GABA–benzodiazepine complex. The site at which barbiturates act is regulated by chloride ions and may be part of the chloride ion channel protein where GABA elicits synaptic inhibition by increasing ion permeability. Numerous drugs with nonbenzodiazepine structures mimic benzodiazepines pharmacologically and at receptor-binding sites. Most of these agents act at the same recognition site as conventional benzodiazepines. The nonbenzodiazepine agents suriclone and zopiclone act at yet another site distinct from the GABA, benzodiazepine, and barbiturate recognition sites but this site does modulate benzodiazepine binding.

Receptor-binding techniques have been applied to sites other than those involved in neurotransmitter recognition. For instance, direct studies of binding of toxins to ion channels, especially the sodium ion channel, have been carried forward by Catterall and other groups. Sodium channel proteins have been isolated and some amino acid sequences identified, permitting an attack on gene cloning of channel proteins. Evidence for selective ion influences on neurotransmitter binding in the

central nervous system dates back at least to 1974, when chloride ion in physiological concentrations was found to modulate glycine receptor binding. Synaptic hyperpolarization produced by glycine is associated with enhanced chloride ion permeability. A variety of anions can mimic these actions of chloride, whereas some anions fail to penetrate the chloride ion channel. Those ions that can mimic chloride neurophysiologically also mimic its ability to influence glycine receptor binding; anions that fail to traverse the chloride channel also do not affect glycine receptor binding.

More direct studies of ion channels related to drug action have proliferated in studies of receptor sites for calcium antagonist drugs. At least two distinct receptor sites can be differentiated. One interacts selectively with dihydropyridine calcium antagonists, e.g., nifedipine, whereas another primarily recognizes drugs of the verapamil and diltiazem classes. The two calcium antagonist drug receptors interact allosterically. Autoradiographic studies indicate that the dihydropyridine sites labeled by [^{3}H]nitrendipine are associated with specific synaptic areas and rarely with blood vessels. This suggests that the drugs are not binding to the calcium channel itself. Instead, they interact with a site that possesses many properties of traditional neurotransmitter receptors, which is linked to voltage-dependent calcium channels. The obvious implication is that there might exist a normally occurring neurotransmitter, hitherto unknown, that interacts physiologically with the calcium antagonist drug receptors. The recent development by the Bayer company of dihydropyridines with calcium agonist properties favors such a concept. Such a hypothetical neurotransmitter would be localized to such areas as the limbic system in which receptors for calcium antagonist drugs are concentrated. Actions on neurons possessing such receptors may be important for the regulation of emotions.

Another novel type of receptor studied by binding techniques is the recognition site for neurotransmitter uptake. Neurotransmitter uptake investigations traditionally have monitored the accumulation of radiolabeled transmitter into tissue slices or synaptosomal preparations of the brain. Studies in several laboratories have shown that [^{3}H]imipramine binding to brain membranes labeled sites linked to the serotonin neuronal uptake system. [^{3}H]Desipramine binding can be used to label specific uptake sites for norepinephrine. Uptake sites for dopamine can be labeled with the anorectic drug [^{3}H]mazindol. Similar to the more conventional neurotransmitter receptors, these uptake receptors also display striking ionic regulation. The serotonin, dopamine, and norepinephrine uptake receptors have an absolute sodium requirement. Earlier studies of serotonin and norepinephrine accumulation had shown a dependence on sodium ion thought to reflect a link to the sodium-potassium-ATPase membrane ion pump. The influence of sodium ions on [^{3}H]imipramine and [^{3}H]desipramine binding seems unrelated to the sodium-potassium-ATPase pump. Thus the binding protein of the amine uptake mechanism may have its own ion regulatory site.

All of these advances have placed receptor research at the forefront of molecular neuroscience. Genetic engineering techniques are finding some of their most far-reaching applications in explicating how neurotransmission is effected through receptors. In the eminently practical area of drug development, simple, sensitive, specific assays for many receptor sites are facilitating the emergence of more specific and safe therapeutic agents.

Solomon H. Snyder
Departments of Neuroscience,
Pharmacology and Experimental Therapeutics,
Psychiatry and Behavioral Sciences
The Johns Hopkins School of Medicine
Baltimore, Maryland

Contents

Neurotransmitter Receptor Binding, 2nd ed.,
edited by H. I. Yamamura et al.
Raven Press © 1985.

1

Receptor Models and the Action of Neurotransmitters and Hormones: Some New Perspectives

Morley D. Hollenberg

Endocrine Research Group, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

I. INTRODUCTION

The study of the mechanisms whereby a variety of neurotransmitters, hormones, and drugs activate processes in their target tissues has undergone a veritable metamorphosis over the past decade. (In this chapter the terms *neurotransmitter, drug*, and *hormone* are used interchangeably to denote the ligands with which receptors interact.) The "receptors" for neurotransmitters are membrane-localized proteins that have the *dual* job of recognizing a ligand with exquisite sensitivity and chemical selectivity and then converting the process of recognition into a signal that results in cellular activation. This dual function required of receptors for neurotransmitters and other analogous hormones distinguishes these pharmacological receptors from other highly specific cell surface recognition molecules that may subserve other cellular functions, such as the transport of nutrients or the selective pinocytotic uptake of carrier-bound cell regulators (e.g., the carrier-mediated uptake of iron via transferrin and the transferrin acceptor). Recognition molecules that mediate the uptake of ligands such as transferrin or low-density lipoprotein have been termed acceptors, or receptors, of the "class II" type (69,86).

In order to account for the dual recognition–activation function of receptors (as opposed to acceptors), a number of models have been developed that relate receptor occupation to the generation of a cellular signal. These models were developed initially on the basis of "traditional" pharmacological observations (dose–response curves, structure–activity relationships). However, the comparatively recent acquisition of a wealth of biochemical data has led to re-evaluation of some of the older models of receptor function and has resulted in newer versions of these models.

The new models describe receptor function in molecular terms, involving multiple interactions among membrane-localized macromolecules. The recently developed models also take into account a variety of dynamic processes in which a receptor may participate in the course of cell activation.

This chapter attempts to provide a perspective for interpreting several of the models of receptor function that relate to the action of cell-surface receptors and to give the reader a starting point, both conceptually and with reference material, for understanding the numerous receptor models that have been developed in detail in other publications. For detailed mathematical treatments of some of these models and for more in-depth descriptions of the models, the reader is strongly encouraged to consult the reference material and the recommended reading list at the end of this chapter.

Unfortunately, a complete description in mathematical terms of all the models considered here is beyond the scope of this chapter. No attempt is made to present each model in complete detail or to comprehensively review all models of drug action that have been described in reviews (3,8,14,26,35,47,70,76,77,87,90–92,94,105,113,114) and in three comparatively recent, very useful treatises (11,85,112). Rather, this chapter presents an overview of selected models of drug

action that have been and should continue to be of value in planning an experimental approach to the study of neurotransmitter action.

In large part, progress in the understanding of receptor mechanisms has come from the development of reliable methods for measuring the receptor-specific binding of radiolabeled ligand probes (either antagonists or agonists). As described in this volume and elsewhere (8,9,35,54,55,59,67,76,77,94,112), such studies have led to the accumulation of a vast amount of data about many receptors, including their molecular weights, subunit compositions, and amino acid sequences (so far, nicotinic cholinergic receptor only), the kinetics of receptor turnover, the phosphorylation of receptor substituents, and the localization of receptors at the cellular and subcellular level. The challenge that faces us is to integrate the empirical information now available into a mechanistic construct that can explain in detail the manner whereby ligand recognition is translated into an integrated cellular and tissue response.

II. DOSE–RESPONSE CURVES AND THE RELATIONSHIP BETWEEN LIGAND BINDING AND BIOLOGICAL RESPONSE

A. General Considerations

The concept of a receptor is inextricably linked to the biological response caused by a neurotransmitter or hormone of particular interest. Indeed, before 1965 practically all of the information about receptors was deduced from an analysis of dose–response data. Because it was clearly understood, as stated explicitly by Ehrlich, that for an agent to act it must be bound, the first receptor models focused on the binding function of the receptor. Accordingly, it was assumed (26) that the interaction between a drug such as a neurotransmitter (H) and its receptor (R) was a reversible bimolecular reaction described by the equation

$$H + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH \quad \rightarrow \rightarrow \text{response} \qquad [1]$$

where k_1 and k_{-1} represent the forward and reverse rate constants, and the quotient k_{-1}/k_1 equals the equilibrium dissociation constant K_D.

This simple equilibrium, which assumes that all receptors in the system are *equivalent* and that all receptors bind independently of one another, forms the basis of a number of models of drug action. These models focus either on the equilibrium concentration of the neurotransmitter–receptor complex (occupancy models) as developed by Clark (22–26), Gaddum (44,45), Ariens (1,2), Stephenson and co-workers (104,105), and others, or on the rates of formation and dissociation of the ligand–receptor complex, as emphasized by the "rate theory" developed by Paton and co-workers (87–89). Note that Clark, the first proponent of the occupancy model, also fully appreciated the mechanistic implications of the rates of drug action (26). Thus the "occupancy" models of receptor function are every bit as

concerned with the rates of the reactions proposed as is the "rate model." Although, as outlined below, the rate theory and occupancy theories differ with regard to the immediate function of the ligand–receptor complex, both theories consider the complex as a distinct physicochemical entity, with properties different from those of the uncomplexed components. Thus as further details become available concerning the biochemical events leading to cellular activation by neurotransmitters, the apparent differences between the rate and occupancy theories may become somewhat arbitrary; that is, on the one hand, rapid rates of ligand-mediated activation, associated with the initial formation of the hormone–receptor complex, must be explained by the occupancy models; and, on the other hand, the series of biochemical reactions in which the ligand-occupied receptor participates must be dealt with by the rate theory, which postulates that it is only the *initial* formation of the ligand–receptor complex that generates a signal (*see below*). The occupancy model (for an elegant mathematical treatment of this model, see ref. 11) and the rate theory form an important point of departure for many of the mathematical models of drug action.

B. Equations Based on the "Occupancy" Assumption

One tenet of the occupancy theories is that the magnitude of the biological response is in some way proportional to the amount of ligand–receptor complex formed: [RH]. If it is assumed that the drug–receptor interaction is bimolecular and reversible, as given by Eq. 1, at equilibrium, according to the law of mass action, then the dissociation constant K_D of the ligand–receptor complex is given by

$$K_D = ([H]\,[R])/[RH] \qquad [2]$$

where [H], [R], and [RH] represent the concentrations of *free* hormone, receptor, and receptor–hormone complex, respectively.

In its simplest form, as developed by Clark (22–26) and Gaddum (44,45), the occupancy theory assumes that the concentration of receptor–hormone complex [RH] is directly proportional to the pharmacological effect (Q)

$$Q = \alpha[RH] \qquad [3]$$

where α is a proportionality constant. Furthermore, in the simplest case it is assumed that unoccupied receptors elicit no response and that the maximum response (Q_{max}) is attained when all receptors are occupied; that is

$$Q_{max} = \alpha[R_T] \qquad [4]$$

where $[R_T]$ is the total receptor concentration, given by the sum of free and complexed receptor

$$[R_T] = [R] + [RH] \qquad [5]$$

Substituting Eq. 5 into Eq. 2 and rearranging

$$[RH]/[R_T] = [H]/(K_D + [H]) \quad [6]$$

Dividing Eq. 3 by Eq. 4

$$Q/Q_{max} = [RH]/[R_T]$$

Thus it follows that

$$Q = Q_{max}[H]/(K_D + [H]) \quad [7]$$

Eq. 7, derived from the initial assumptions, indicates a hyperbolic relationship between the response and the *free* concentration of hormone. The concentration of hormone producing a half-maximal response, $[H]_{0.5}$ (usually termed the ED_{50}), can be equated with the equilibrium dissociation constant for the hormone–receptor interaction.

The form of Eq. 7 is identical to that of the well-known substrate–velocity relationship of enzyme kinetics; Q_{max} in Eq. 7 represents the more familiar V_{max}, and Q replaces V, the reaction velocity. It is thus possible to transpose many of the mathematical relationships of enzyme kinetics developed for enzyme substrates (drug agonists) and enzyme inhibitors (drug antagonists).

In addition to the assumption of proportionality between occupancy and response as indicated by Eqs. 3 and 4, Eq. 7 presupposes a *simple* bimolecular reaction between a hormone and a unique receptor binding site that (a) does not interact with other binding sites and (b) does not change its binding characteristics upon being occupied by its specific ligand. In practice, dose–response curves usually depict response versus a function of $[H_T]$, the total concentration of drug present in the assay system, on the assumption that the proportion of bound drug is very small, such that $[H_T] \simeq [H]$, the free drug concentration. Should an appreciable fraction of drug be bound, the total concentration of hormone, $[H_T]$, at which the response is 50% of maximum will exceed the K_D by an amount equal to one-half the total concentration of receptor, as indicated in the following derivation.

If $f\ (0 < f \leq 1)$ represents the fraction of the maximum response at a given hormone concentration

$$f = Q/Q_{max} = [RH]/[R_T] \quad [8]$$

then from Eq. 6

$$f = [H]/(K_D + [H]) \quad [9]$$

or

$$[H] = K_D[(f)/(1 - f)] \quad [10]$$

Because $[H_T] = [H] + [RH]$ and from Eq. 8 $[RH] = f[R_T]$, substituting into Eq. 10 gives

$$[H_T] = K_D[f/(1 - f)] + f[R_T] \quad [11]$$

It thus appears that under certain experimental conditions receptor concentration can become an important consideration for the analysis of dose–response data, jus

as enzyme concentration may prove to be a factor in the analysis of substrate–velocity relationships, as emphasized by Straus and Goldstein (107). When $[R_T]$ is very small (i.e., the concentration of bound drug, $f[R_T]$, is negligible), then

$$[H_T] = K_D[f/(1 - f)] \quad [11a]$$

and at the half-maximal response, $f = 0.5$ and thus $[H_T]_{0.5} = K_D$. If R_T becomes very large with respect to the K_D, then in the extreme

$$[H_T] = f[R_T] \quad [11b]$$

and the total drug concentration is linearly related to the fractional response. From Eq. 11 we see, as indicated above, that the total concentration of ligand at which response is half-maximal is given by

$$[H_T]_{0.5} = K_D + 0.5[R_T] \quad [11c]$$

The same considerations concerning receptor concentrations apply to the analysis of ligand-binding data (Chapter 3), as dealt with in detail by Rodbard (91).

For many pharmacological studies with nerve and muscle preparations and with isolated cells, e.g., adipocytes, the concentration of receptors is indeed well below the estimated K_D of the receptor–ligand interaction. For instance, insulin-mediated enhancement of glucose transport in adipocytes is half-maximal at an insulin concentration of approximately 3×10^{-11}M. From the available experimental data (approximately 2×10^5 cells per milliliter used in an assay, with 10^4 receptors per cell) (32), the receptor concentration can be estimated to be 3×10^{-12}M. The $[H_T]_{0.5}$, or ED_{50}, for insulin action might thus be taken as a theoretical estimate of the receptor K_D for insulin, based on bioassay measurements, neglecting the last term of Eq. 11 and assuming a linear occupancy–response relationship, i.e., assuming that Eqs. 3 and 4 are true.

The data in Fig. 1 illustrate some of the considerations discussed above. Curves *a* to *f* represent theoretical curves based on Eq. 11 for different concentrations, $[R_T]$, of a receptor possessing a hypothetical ligand dissociation constant of 5×10^{-11}M. Curve *a* represents Eq. 11a; curve *f* approximates Eq. 11b. The right-hand ordinate, *f*, can be taken to represent either fractional binding of a ligand to a receptor or the fractional biological response. The difficulty in interpreting an ED_{50} without knowledge of either the free concentration of hormone or the total concentration of receptor, $[R_T]$, is apparent in Fig. 1.

Superimposed on the theoretical curves in Fig. 1 are dose–response and receptor-binding data obtained in replicate human fibroblast monolayers (i.e., R_T is the same for both measurements) for the polypeptide epidermal growth factor-urogastrone (EGF-URO) (18,58,60). [EGF-URO is a mammalian polypeptide that was found and characterized initially in the mouse and is now known to be present in humans, many other mammals, and several lower vertebrates. This polypeptide of about 6,000 daltons is a potent stimulator of cell growth and an inhibitor of gastric acid secretion in mammals (17,52,57).] A receptor concentration of approximately