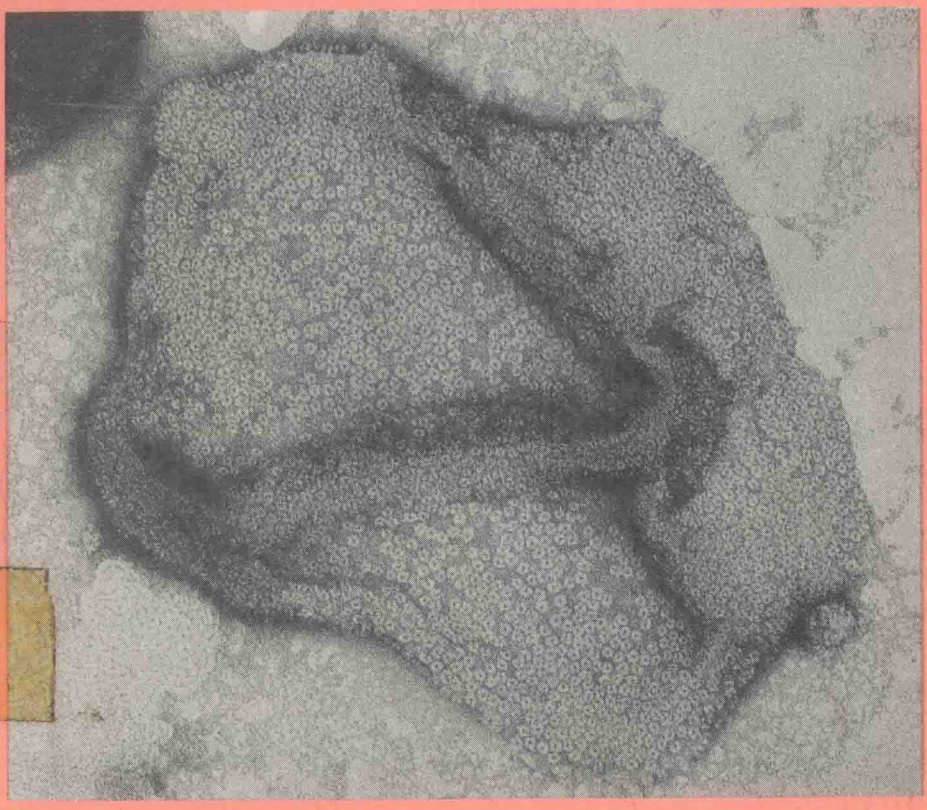
More About Receptors

Current Reviews in Biomedicine 2

Foreword by A. W. Cuthbert

Edited by John W. Lamble



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More About Receptors

Foreword

There is nothing, so they say, that succeeds like success. The first collection of papers selected mainly from *Trends in Pharmacological Sciences (TIPS)* and entitled 'Towards Understanding Receptors' which was issued in 1981 proved to be such a successful venture that there is little need to introduce this second collection. The format for 'More About Receptors' is precisely the same as for the former volume, that is, selected, unexpurgated articles from *TIPS* and its sister journals have been arranged in a sensible order and recast in textbook format.

TIPS articles are not reviews in the strict sense and authors are allowed, or even encouraged, to present their own point of view developed from results gathered in their own laboratories. No Tennyson's 'chorus of indolent reviewers' are these writers but hard-headed, practical scientists with a story to tell. Information about receptors is now accumulating at such a rate that pharmacologists need a newspaper to keep abreast of the developments in fields not their own. *TIPS* fills this need and collections of articles, such as this, provide a very convenient format with all the clutter removed. There are, however, some dangers of collecting the articles together between boards, be they hard or soft. It provides an illusion of permanence for what is a collection of 'state of the art' articles. The ephemeral nature of many of the hypotheses will doubtless be revealed in editions of *TIPS* yet to come, and volumes like this one will chronicle the evolutionary paths of modern pharmacology.

Few pharmacologists will find nothing to interest them in this collection, while most who are concerned with the molecular aspects of receptors will wish to keep the volume close by. The succinct style of the articles, not overburdened with references, make it a very handy book for both graduate and undergraduate students, and keen-eyed examiners will have noticed that student essays can have a more than superficial resemblance to *TIPS* articles.

Two articles on membrane receptors and hormone action by Hollenberg, the first dealing with structure and regulation and the second with function, provide a convenient opening scenario. Indeed, so wide is the coverage in these chapters that they could have served as an appropriate foreword to the whole collection. In an article I wrote in the inaugural issue of *TIPS*[1] I suggested that progress in understanding receptors had been hindered by the ease with which drug responses can be recorded using simple physiological systems, leading to an excessive concern with 'autonomic' receptors. These seemingly straightforward measurements were, of course, very far removed from the actual interactions between drug and receptor. Hollenberg's articles show just how far progress has been made and it is refreshing that receptors for, say, epidermal growth factor are mentioned alongside nicotinic receptors, even though the 'response' to activation of the former is complex and ill-understood. What is important to Hollenberg is the receptor, what it is made of, how it works and is regulated. One cannot help but feel hopeful that general principles about receptor mechanisms will eventually emerge from the mass of information now being collected. One principle that is fast becoming established as a general mechanism of receptors relates to their dynamic nature, a theme pursued in some detail in a later contribution by Perkins. Receptor properties and membrane densities are now known to change in response to a variety of perturbations with, of course, dramatic

consequences for the relationship between drug concentration and response. The need to make measurements as close as possible to the receptor events is ever pressing; ligand binding and patch clamping are techniques which have already contributed much in this respect. At the end of the second article by Hollenberg some of the questions for future research are posed. The techniques that will be required are to some extent obvious and pharmacologists must do what they always have done, that is, borrow them from other disciplines if they are appropriate.

One way in which receptor occupation can be translated into a cellular response is via adenylate cyclase, which properly continues to occupy a front-line position in receptor research. Several papers on this topic are included here. Levitzki describes the intricacies of the interactions between receptor, GTP binding protein and the catalytic subunit of adenylate cyclase. Another paper, by Porzig, points to the importance of cellular studies as well as the 'grind and bind' approach, highlighting important differences which have arisen from experiments in the β-receptor-adenylate cyclase system of whole cells and homogenates. 'Anti-idiotype' antibodies (anti-antihormone antibodies) have been used by the immunology group of CNRS in Paris to look at the β-receptor-adenylate cyclase complex. This is a novel approach which has also been applied to the insulin receptor and it will be interesting to see how far this technique can be applied to other systems. In general, pharmacologists have been rather slow to exploit immunological techniques for receptor problems. Development and use of monoclonal antibodies and anti-antihormone antibodies for the demonstration, localization and isolation of membrane receptors are obvious ways forward. An article from *Immunology Today*, a sister publication to *TIPS*, is included in this collection for those who may wish to become a little more familiar with these approaches.

The role of cAMP in the nervous system and in synaptic transmission is considered in two other contributions. It is pointed out that there are many steps in the process between cAMP generation and the pharmacological response, involving, for example, cAMP-dependent protein kinases or protein phosphatases, which have yet to be targets for drug action. Recent reports on the actions of the diterpene forskolin, apparently a direct activator of the catalytic subunit of adenylate cyclase[2], are perhaps an indication that other steps in the cascade, beyond the initial recognition events, are pharmacologically amenable.

It is intriguing that in the adrenoceptor area the variety of drugs available and their specificity seems inversely related to the period of study. Only one paper is included on α-receptors, by Exton. He concludes that the presynaptic α_2-receptors are operated by unknown molecular mechanisms, post-synaptic α_2-receptors inhibit adenylate cyclase by uncertain mechanisms while post-synaptic α_1-receptors may operate through activating calcium gates or the ubiquitous phosphatidylinositol (PI) mechanism. On the other hand, for the dopamine receptor, which is quite the baby of the adrenoceptor family, one is spoilt for choice. It is proving difficult to classify dopamine receptors, or even it seems, to decide a basis for classification. Three papers on this topic are included in this volume, and while there is a deal of common ground between them there are subtle differences. It is right that the reader should be exposed to the three points of view and, furthermore, all the three articles by Cools, by Beart and by Offermeier and van Rooyen should be consumed at one reading.

Very low concentrations of apomorphine inhibit dopamine synthesis in the striatum and limbic system. This paradoxical effect has been linked to the activation of dopamine (presynaptic) autoreceptors. Nilsson and Carlsson describe in their article the actions of a

new compound, 3PPP, which is apparently highly specific for dopamine autoreceptors and which fails to have post-synaptic actions at near lethal doses. The possibility of obtaining selective antipsychotic actions without troublesome extrapyramidal side effects is exciting.

No collection of articles on membrane receptors would be complete without something about GABA receptors. The relation between benzodiazepine binding sites and GABA receptors is well known and in his article Möhler considers whether or not there are endogenous ligands for the binding sites for benzodiazepines. Barbiturate drugs also affect the interaction of GABA with its receptors and Johnston and Willow propose that the barbiturate receptor may be a lipid, in their article on barbiturate receptors.

The ways in which muscarinic acetylcholine receptors are coupled to their final effector process is unclear. It has, of course, been known for 20 years or so that activation of muscarinic receptors in different situations can lead to very different biophysical events, for example, an increase in potassium ion permeability leads to hyperpolarization in the heart while in smooth muscle a depolarization results from an increase in permeability to sodium and/or calcium ions. Also, the response to muscarinic receptor activation is relatively slow suggesting that, in some instances at least, coupling via metabolic processes is required. This theme is pursued by Hartzell in his paper on the physiological consequences of muscarinic receptor activation.

It is to be remembered that the first detailed ligand binding study was made on the muscarinic receptor using ^{14}C-labelled atropine[3]. A variety of reversible ligands with affinities greater than those of atropine are now available as well as rather specific irreversible compounds such as the benzilylcholine mustards. A view of the muscarinic receptor gained from ligand binding studies is given in a paper by Sokolovsky and Bartfai, one of two papers in this volume taken from *Trends in Biochemical Sciences (TIBS)*.

No matter where they are located, nicotinic receptors when activated cause a rapidly developing, short lasting increase in membrane permeability with very little ion selectivity. It seems likely, therefore, that the recognition site of the nicotinic receptor is closely associated with a membrane ionophore. As a consequence it is feasible to examine both binding studies and simultaneous ion fluxes by use of a suitable membrane vesicle preparation. This is precisely what Taylor and Sine have done in order to study the relation between occupancy and activation of the permeability mechanism.

Non-imidazole histamine H_2-antagonists are the subject of the only paper on histamine receptors in this volume.

Ever since it was found that adenosine could modify adenylate cyclase activity the possibility of the existence of physiologically relevant adenosine receptors has been explored. What has been learned from direct binding studies using non-metabolizable ligands is reviewed by Schwabe.

Although no papers on the insulin receptor were included in 'Towards Understanding Receptors', results with this receptor continue to raise intriguing questions such as, 'why is the insulin receptor complex internalized?', 'is internalization essential for coupling the membrane event with the effector system?' and 'is the insulin receptor complex responsible for directing the insertion of glucose transporting activity from the Golgi to membrane and, if so, what are the second messengers?' The earlier omission has been rectified by including a paper on insulin action and one on structural features of the receptor, the second of these coming from *TIBS*.

The involvement of membrane lipids in drug action is not a new concept but their precise role is yet to be formulated. A paper by Cockcroft further explores the Michell

hypothesis that breakdown of PI as a result of receptor activation is responsible for calcium gating. One cannot help but feel a sense of *déjà vu* when reading the literature on PI breakdown and I had cause to reread what I had written on this topic in a review in 1967[4]. The Hokins had done much to establish the mechanism of drug-mediated PI turnover in the mid-1950s and had suggested that it might be involved in sodium ion translocation. Indeed it was a disappointment to find that oxytocin, which stimulates sodium transport in toad bladder, does not cause ^{32}P incorporation into phospholipids. On the other hand, acetylcholine did stimulate ^{32}P incorporation but was not shown to stimulate sodium transport[5]. Now it is recognized that the oxytocin effect is mediated by cyclic AMP, and no other receptor known to stimulate adenylate cyclase has been shown to stimulate PI turnover. Furthermore, high-resistance sodium transporting epithelia have now been shown to respond to acetylcholine under appropriate circumstances[6]. Thus some of the data, which led earlier investigators to abandon the idea that PI turnover was associated with sodium ion translocation, can now be seen to fit well the criteria of the Michell hypothesis. Nevertheless the question still remains one of cause or effect. Cockcroft points out that ATP-induced histamine secretion from mast cells is calcium dependent but, in the absence of calcium, the PI response does not occur. In this instance it would seem that the PI response is an effect, rather than the cause of the calcium influx! More recent studies from Michell's laboratories have concentrated on the reactions of triphosphoinositides with impressive calcium chelating activity. Doubtless a later collection of papers from *TIPS* will have a contribution on this topic.

Histamine release from mast cells is also the subject of the paper by Axelrod and Hirata. Concanavalin A stimulation of mast cells leads to the progressive methylation of phosphatidylethanolamine to phosphatidylcholine, an event which precedes both Ca^{2+} influx and histamine release. Inhibitors of the methyltransferases involved in the methylation reactions also inhibit Ca^{2+} influx and release of histamine. These authors have developed a particularly elegant way of demonstrating the importance of the transferase reactions without the use of drugs. Rat basophilic leukaemia cell lines were isolated which were deficient in either methyltransferase I or methyltransferase II activity and unable to release histamine with the usual stimuli. After the cells were fused to form a hybrid, histamine releasing capacity was restored. A full account of this approach has now appeared[7].

The last paper in this volume presents an unusual riddle. Using the guinea-pig papillary muscle Ebner has demonstrated a propranolol resistant inotropic effect which is blocked by hydrocortisone. Arguing from the known effects of these drugs the author is forced to conclude that part of the inotropic effect comes from activation of intracellular receptors. Receptors, like other membrane macromolecules, must be synthesized by the cell and inserted into the membrane after passing through the usual processing arrangements. The possibility that receptors can be activated before insertion in the cell membrane and, more mysteriously, be coupled to an effector process does not fit within the present framework of thinking about adrenoceptors. Clearly there is a challenge here for future authors of *TIPS* articles to pursue these interesting findings.

It has been a pleasure for me to reread these articles for inclusion in this volume. G. Alan Robison wrote in the foreword to the first volume, 'many authors have let their interest and exitement show through'; this is no less true of this collection. It is fun to read and, in my view, a true picture of the 'state of the art'.

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Cambridge

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Preface

This second volume of papers about receptors, selected from Elsevier magazines, appears less than 12 months after the first. Growth of knowledge in this area is tremendous and for this reason alone a second book would be justified. However, it should also be stressed that *More About Receptors* and *Towards Understanding Receptors* are largely complementary, and many themes appear in this volume which were not covered before.

Although most papers reproduced here have been taken from *Trends in Pharmacological Sciences*, two come from *Trends in Biochemical Sciences* and one comes from *Immunology Today*. This emphasizes that it is topics like receptor studies, in which various disciplines mingle and cross-fertilize each other with paradigms and techniques, which represent the most dynamic growth areas of science. One inhibitor of such interactions is the specialized jargon which permeates so much scientific literature. It is thus a pleasure to report the efforts of authors whose papers are published here, to minimize this feature and thus provide much enjoyable reading.

I am glad, also, to express my deep gratitude to Professor A. W. Cuthbert for his advice during the assembly of the book and for his contribution of an excellent foreword.

JOHN W. LAMBLE

Contents

Membrane receptors and hormone action I:

new trends related to receptor structure and receptor regulation

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Introduction

Along with other rapidly developing areas of cell biology, the study of receptor*-related mechanisms that lead to cell activation is undergoing a veritable metamorphosis. Great strides have been made since the development of the receptor concept at the turn of the century (from 'fiction, to fact', as detailed elsewhere[1,2]), to the point where biochemical details about several of the protein oligomers responsible for ligand recognition are becoming available; and where for certain systems, the individual membrane-localized reactions that occur subsequent to ligand binding are being elucidated. It would be impossible for a single review to document with justice all of the very many exciting studies that are continually appearing to describe the molecular characteristics of receptors for an ever widening number of ligands; almost without exception, the study of each receptor yields some surprises and brings to light more challenges. Rather, an attempt will be made to use selected examples of studies that illustrate the kind of progress that has been made over the past few years and that point to new areas of potential development. In this first article, the focus will be on receptor structure and receptor regulation; a subsequent article will deal with receptor-modulated cell function. For reasons of economy, attention will be focused on those receptors that are localized in the plasma membrane.

The status quo

Before proceeding, it is useful to give a synopsis of the present picture of membrane receptor function. Methods are well in hand for receptor identification, both by pharmacological and biochemical (primarily ligand binding) methods[3]. To date, most of the recognition molecules appear to be rather large oligomeric protein† species, that in detergent solution exhibit mol. wts in the 100,000–300,000 range; the size of the individual recognition subunits varies from about 40,000 (acetylcholine receptor) to about 180,000 (receptor for epidermal growth factor-urogastrone). The receptors appear to be in a dynamic rather

* The term receptor is used here in a restricted sense to denote a molecule that exhibits the dual function of ligand recognition and cell activation.

† Note however the ability of small non-protein molecules, e.g. gangliosides, to function as binding substituents or receptors for certain toxins and hormones[4].

than a static state, both in terms of the turnover of these membrane-localized substituents (*de novo* synthesis, insertion, internalization and 'processing') and in terms of the mobility of certain receptors within the plane of the plasma membrane. The mobility of certain receptors may be relatively restricted (e.g. those, like the acetylcholine receptor that modulate a localized ion channel), whereas other receptors (e.g. those that modulate adenylate cyclase) appear to have comparatively unrestricted mobility, so as to permit a variety of interactions with other membrane-localized substituents. There are a number of theories of receptor function (reviewed in some depth elsewhere[1–3,5]) that take into account both receptor mobility and the multipoint/allosteric nature of ligand binding. In essence, the functions of receptors like other oligomeric proteins can be understood in terms of regulatory mechanisms that have been observed to operate in a variety of multi-enzyme systems; the challenge lies in finding the appropriate enzymatic paradigm that best matches the idiosyncracies of a particular receptor system. This is not to say that the documentation of new receptor mechanisms will not require novel insight, but rather that the recognized enzyme-like nature of receptor interactions provides a rich point of departure for those facile with complex enzymatic mechanisms. In brief, many membrane receptors can be viewed as complex, mobile enzyme-like oligomers that function in a restricted membrane environment.

While the above synopsis may be an oversimplification of an 'obvious' state of affairs, it is fair to say that it has taken about 80 years to arrive at the point where this explicit statement can be made with any confidence (notwithstanding the recognized shortcomings of our present understanding). Where, then, does one go from here? On a comprehensive 'wish list', one would aim for more in-depth studies of several selected receptor systems. More information is needed about the molecular structure of receptors (sequence, common regions in different receptors, overall conformation), about functions that govern receptor regulation (both at the genetic and epigenetic level) about the kind of information stored in the receptor *per se*, about the detailed series of protein–protein interactions that lead from ligand recognition to a specific cellular response, and about the relationship of receptor structure and function to the pathogenesis of certain disease states. The lessons learned about hormone receptor structure and function may well apply to other membrane constituents (antibodies, cell–cell recognition sites) that modulate cellular function.

Receptor structure

Except for the nicotinic receptor for acetycholine, the amounts of receptor material available for study usually preclude the use of direct chemical analysis (amino acid analysis, sequence, etc.). Nonetheless, a great deal of information has been obtained with the use of the specific high affinity ligands as receptor 'markers', along with photoaffinity and affinity-crosslinking labeling methods, enzymatic probe techniques and lectin-probe methods. Thus, data obtained for receptors for polypeptides such as insulin or epidermal growth factor-urogastrone (EGF-URO) indicate that the receptors are glycoproteins that are only partially embedded in the membrane lipid environment. It is interesting to note that important functional information resides both in the protein and non-protein constituents of receptors. It is now evident, for instance, that the oligosaccharide portion of receptors may play a role both in the ligand recognition function (e.g. removal of sialic acid augments the binding of EGF-URO to its receptor) and in the signal-transduction process (e.g. neuraminidase abrogates insulin action in adipocytes without affecting insulin bind-

ing). A contribution of other non-protein moieties (possibly, tightly receptor-associated via non-covalent mechanisms) to ligand recognition can be seen in the likely participation of gangliosides in the binding and action of agents such as thyroid stimulating hormone and interferon[4].

In terms of protein structure, new data for the insulin receptor indicate that two or more polypeptide chains appear to be involved in ligand recognition (data summarized in several communications in Ref. 6). On the one hand, photolabeling and affinity crosslinking methods have been used in the laboratories of M. Czech, S. Jacobs, M. Wisher, C. C. Yip and others[6], to identify a ligand recognition species (alpha-chain) that upon electrophoresis under reducing conditions exhibits a mol. wt of about 135,000; electrophoresis under non-reducing conditions indicates that the labeled constituent behaves as a species with a mol. wt somewhat above 300,000. Crosslinking experiments, using disuccinimidyl suberate[6] indicate that, upon binding to the receptor, insulin is also in proximity to a second polypeptide (beta-chain) that, under reducing conditions, has a mol. wt of about 90,000. Limited reduction of the insulin-labeled receptor yields a species with a mol. wt in the 200,000 range. On the other hand, these data obtained by ligand-crosslinking methods are complemented by the detection of similar protein constituents, upon analysis of insulin receptor that has been highly purified by affinity-chromatographic methods[6]. Taken together, the data indicate an oligomeric structure of the insulin receptor, with a two-chain ligand recognition species ($\alpha\beta$) that may exist in the membrane as a disulfide-linked multimer $(\alpha\beta)_2$. For insulin, the ligand recognition event may turn out to be a very complicated process, including not only the participation of a number of polypeptide chains comprising the receptor recognition oligomer *per se*, but also including input from other 'non-recognition' or 'non-receptor' glycoprotein moieties with which the receptor oligomer can interact.

Even more detailed information about receptor structure–function relationships can be anticipated from studies with the nicotinic-cholinergic receptor[7], for which substantial amounts of material can be obtained from *Torpedo* and *electroplax* species. In detergent solutions, the receptor behaves as a species with an apparent mol. wt of about 250,000; the entire oligomeric structure ($\alpha_2\beta\gamma\delta$) comprises two recognition subunits (α, mol. wt about 40,000) and an oligomer ($\beta\gamma\delta$) (possibly the ion channel) composed of three distinct, but chemically-related[8] substituents with mol. wts of about 48,000 (β), 58,000 (γ) and 64,000 (δ)[7]. As the detailed structures of the ligand recognition components and the ion channel species become available, it should be possible to improve our understanding of the complex interactions of agonists, partial agonists, antagonists and ion-channel-specific agents that modulate the ion transport function of this complex oligomeric receptor.

Receptor regulation

It is now apparent, as reviewed in some detail elsewhere[2], that cell surface receptors for a variety of agents can be modulated at one of several levels. In cells like quiescent lymphocytes, cell activation (antibody, plant lectin) can lead to the *de novo* appearance of receptors, such as the one for insulin. Further, the appearance of specific receptors, and the coupling of receptors to effector systems (e.g. adenylate cyclase, cation transport systems) can be observed to be developmentally related. For instance, in mouse embryos, receptors for epidermal growth factor increase during gestation, with a pronounced effect evident in a target tissue such as the maxilla[9]. Similarly, for β-nerve growth factor in the chick, receptor content and β-nerve growth factor responsiveness vary with

development. In maturing rat erythrocytes, catecholamine responsiveness (adenylate cyclase) decreases markedly, without concomitant changes in the erythrocyte receptor content. Presumably in many instances of this kind related to development, changes in receptor content and function will be found to be hormonally regulated at the genetic level (e.g. steroid hormones can control the receptors for peptide hormones). Apart from changes related to a developmental process, changes in the receptor for one hormone can be regulated by a second hormone. This kind of regulation may be termed 'heterospecific' receptor regulation, in which the process is distinct from the control of receptor internalization (see below)[10]. Heterospecific hormone receptor regulation represents an important control point for a variety of biological processes and, therefore, represents a fascinating area for future research. As nucleic acid probes become available for receptor-related research, it may be possible to study this aspect of the control of receptor biosynthesis at the genetic level. Such studies will complement the methods already available for monitoring receptor turnover (heavy isotope methods) and membrane insertion (conventional ligand binding methods). In brief, given the availability of antireceptor antibodies (see below) and given the recent advances in molecular biology, it would appear that a new threshold of sophistication in the analysis of receptor regulation may be at hand.

Evidence is now emerging for a kind of heterospecific receptor regulation that is distinct from control at the genetic level. The site of this control appears to be localized within the plasma membrane. For instance, work from the laboratories of I. B. Weinstein, G. J. Todaro, E. Rozengurt and others (summarized in Ref. 11) indicates that the tumor promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA), interacting with its own distinct membrane receptor, causes a rapid (tens of minutes) time- and temperature-dependent selective reduction of cell receptors for EGF-URO. Evidence has also been obtained for a heterospecific regulatory interaction (up- and down-regulation) between the receptors for EGF-URO, platelet-derived growth factor and fibroblast growth factor[12]. In contrast, carbachol, acting via a muscarinic receptor in rat cardiac tissue can increase the affinity of the α_1-adrenoceptor for the α-adrenergic antagonist ligand WB4101 [2-N(2,6 dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane][13]; the muscarinic agonist partially reverses the decrease in α_1-receptor ligand affinity caused by the guanine nucleotide analogue, guanyl-5′-imidodiphosphate (GMP-P(NH)P). Another intriguing example of this kind can be seen in the insulin-mediated augmentation of the binding of the insulin-like growth factors, multiplication stimulating activity (MSA) and basic-somatomedin by rat adipocytes[14,15]. For MSA, the increase in receptor binding caused by insulin receptor occupation is due to an increase in MSA ligand affinity; the effect is time- and temperature-dependent and requires an intact cell. A final striking example of membrane-localized heterospecific receptor regulation is evident in the complex reciprocal relationship between the binding of gamma-aminobutyric acid (GABA) and the benzodiazepines in isolated membrane preparations (summarized in Refs 16–18). The binding data are consistent with previously obtained electrophysiological evidence indicating that diazepam augments GABA-mediated inhibitory effects on neurons. The increase in [^{3}H]GABA binding caused by diazepam, and the augmentation of [^{3}H]methyl-diazepam binding in the presence of GABA can be observed within minutes in isolated membrane preparations. The effect of diazepam on GABA binding has been attributed to its ability to

complex with a separate non-receptor endogenous membrane-associated thermostable component (GABA modulin; mol. wt about 15,000) that reduces the GABA receptor's affinity for GABA; the mechanism whereby GABA affects diazepam binding is not known. It is of interest that, upon solubilization of GABA receptor-containing membranes, both the GABA-binding and diazepam binding activities appear to be associated during purification. It is thus possible that in the intact cell, the various components responsible for the complex GABA/diazepam heterospecific reciprocal receptor regulatory process may exist as a large oligomeric structure. The above examples illustrate a relatively recently discovered type of heterospecific receptor regulation. It will be of great interest in future work to look for similar regulatory processes in other receptor systems.

In addition to heterospecific receptor regulation, it is well recognized that certain hormones can modulate the cell content of their own receptors (this can be termed 'homospecific' up- or down-regulation). For instance, brief (tens of minutes) exposure of cells to epidermal growth factor leads to a marked reduction in cellular binding of EGF-URO; the return of cellular binding activity occurs slowly (tens of hours). In contrast, prolactin appears to cause an up-regulation of its own receptors. In the case of EGF-URO, receptor down-regulation can be clearly correlated with receptor occupation. The sequence of events appears to comprise: receptor binding, lateral mobility of receptors to sites of internalization (? coated pits), endocytosis (possibly involving membrane transglutaminase) and lysosomal fusion of internalized receptor-containing vesicles. In the course of internalization, the receptor is subject to proteolysis (or 'processing'); the significance of the internalization process in terms of cell activation is uncertain. Comparatively little is known about the control of the internalization/receptor degradation process. However, it appears that several hormone receptors may be internalized via a similar, receptor-triggered process[10]. Since receptors for insulin, EGF-URO and α-2-macroglobulin can be observed to co-migrate to the same membrane site prior to internalization, one can reasonably predict that on distinct receptor oligomers, common polypeptide regions will be found that participate in the endocytotic process.

There are also non-hormonal factors that modulate cellular receptor function and content. For instance, viral transforming agents or chemicals such as sodium butyrate can cause both qualitative as well as quantitative alterations in cell receptors. Further, the saturation density of cultured cells can modulate receptor content either in a positive or negative direction; the mechanism whereby cell–cell contact alters receptor number will be a fruitful subject for future study. Most interestingly, it now appears that the extracellular matrix upon which cells sit may govern not only cell receptor content, but also may determine the cellular response upon receptor occupation[19,20]. The control of receptor function by matrix constituents (glycosaminoglycans, fibronectin, collagens) provides an intriguing regulatory process, whereby one hormone, acting at its receptor to stimulate cell matrix production can, via an indirect process, modulate the action of a second hormone acting at a distinct receptor site.

From the above discussion, it is evident that the numbers and characteristics of cell surface receptors can be affected by a large number of factors related both to intracellular events (rates of receptor synthesis and turnover, cell cycle, cell differentiation) and to a variety of extracellular stimuli caused by hormones and other agents. These stimuli may be caused either by hormone receptor occupation (homospecific or heterospecific regulation) or by