Review Article

Recent advances in receptor research

Michael Schachter

Department of Clinical Pharmacology, Queen Elizabeth the Queen Mother Wing, St. Mary's Hospital, London, W2 1NY, UK.

History and background

Pharmacology is the science of receptors. This has been appreciated ever since pharmacology began to emerge as a separate discipline. Langley¹ and Ehrlich² proposed the concept that a drug had to interact with a 'receptive substance' and that some form of binding of drug to receptor must occur. This idea was never seriously challenged but, equally, it remained an abstract concept without a physical counterpart. The presence of receptors in a particular tissue could be inferred from the action of drugs on endogenous substances, but nothing was known of the cellular or subcellular localization of the receptor, its structure, or its mechanism of action. In the last 15 years this situation has been totally transformed. As this review will attempt to show, we now have a vast body of knowledge concerning the above questions for most of the 'classical' receptors, and also for many whose existence was not even suspected in the 1970s.

There is no single reason for these spectacular advances, which have parallels in many other branches of the life sciences. The following developments are, however, of major significance.

(i) The discovery of enkephalins and endorphins by Kosterlitz and Hughes.³ Apart from the obvious impact on opioid receptor research, this had wider repercussions. The idea of endogenous ligands for 'drug' receptors was hardly novel: acetylcholine was the earliest example, with the recognition that this endogenous agent recognized muscarinic and nicotinic receptors.⁴ Nevertheless, this discovery greatly encouraged collaboration between pharmacologists, physiologists and biochemists and emphasized areas of common interest.

(ii) The development of radiolabelled ligands for binding studies.^{5,6} This has allowed the identification and quantitation of receptors and their sub-types in small quantities of tissue and, to some extent, permitted their anatomical and subcellular localization.

(iii) The delineation of signal transduction mechanisms for most, though not all, receptors. This has

revealed quite unsuspected metabolic pathways linked to receptor activation, as well as the re-evaluation of known mechanisms.

(iv) As part of the above process, the increasing refinement of electrophysiological techniques for measuring electrical activity and ion flux across the cell membrane.⁷

(v) The extraordinarily rapid advances in the technology of molecular biology. This has already permitted the sequencing and cloning of several receptors.

In this review I will attempt to summarize some of the recent developments in this field. Much of this information is only just being incorporated into undergraduate teaching and may be rather unfamiliar. Needless to say, the primary literature is unmanageably vast. To give some idea of the scale, a review of dopamine receptors in 1980 included over 1200 references.⁸ I will frequently refer to more specialized reviews, especially those in the 'Trends' series of journals.

Radioligand binding

The use of ligand binding techniques is regarded with suspicion by some pharmacologists and physiologists. It is certainly true that 'binding and grinding', as it is disparagingly called, complements but does not replace the classical methods used in pharmacology. In particular, it must always be emphasized that a binding site can only be a receptor if it can be shown to have some functional correlate. If this is borne in mind the techniques of radioligand binding enable researchers to identify, quantify and localize receptors for a vast number of receptors. It is relatively simple and rapid, though seldom cheap. The principle of the technique is in fact very straightforward, and the process is entirely analogous with the reaction of an enzyme with its substrate. The Michaelis constant is replaced by the dissociation constant as a measure of the affinity of the ligand for the receptor, while maximal reaction velocity is replaced by receptor number (the usual abbreviations are K_d and B_{max}).

Correspondence: M. Schachter, B.Sc., M.R.C.P.

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The ligand—usually labelled with tritium or ^{125}I , occasionally with ^{14}C —is incubated with the relevant tissue preparation. The latter may be a very crude homogenate or a more or less purified preparation of a sub-cellular fraction, such as the plasma membrane. ‘Specific’ binding, which may be considered pharmacologically or physiologically relevant, is defined by the subtraction of ‘non-specific’ binding from total binding. Non-specific binding is obtained by co-incubating the labelled ligand with a competing unlabelled, structurally related ligand. Occasionally it may be necessary to use the same ligand in both labelled and unlabelled forms, but this is not ideal. Bound and free labelled ligand are separated at the end of the incubation by filtration or centrifugation: the former is generally more convenient, but centrifugation may be preferable for ligands with relatively low affinity for their receptor. The separation step usually includes a washing procedure (see Figure 1 for a typical scheme). The generally accepted criteria for specific ligand binding are listed in Table I.

Figure 1 Typical scheme for radioligand binding experiment, in this case for labelled neuropeptide Y (NPY) binding to renal cortex. If possible, it is preferable to use a different agent as the unlabelled displacer. However, this is often impracticable for peptides.

Table I Criteria for characterization of receptors using ligand binding experiments

Binding is:	
(i)	saturable
(ii)	reversible
(iii)	displacable by physiologically or pharmacologically relevant agents
(iv)	of high affinity
(v)	correlates with functional response in cell or tissue

Although this technique is very simple in principle it is rarely so in practice. Considerable work is needed to establish the optimal incubation conditions, including ligand concentrations, temperature, method of free and bound ligand separation, and sometimes even the material of the incubation tube. It is easy to get numbers from ligand binding experiments, but great care is needed to ensure that the numbers actually mean something. Analyses of binding data have been subject to well-recognized errors which were the result of attempts to linearize non-linear experimental results, particularly of saturation binding experiments (i.e. those designed to determine receptor number as well as affinity). It is now more usual to use computerized curve-fitting programmes, which make more efficient use of available information.⁹

Binding experiments frequently produce one apparently paradoxical result. The natural ligand for a given receptor may have an apparently low affinity for that receptor. For instance, 5-hydroxytryptamine (5-HT) has a low affinity for the 5-HT₂ receptor.¹⁰ By contrast, antagonists at this receptor, such as ketanserin, have an affinity several hundred times greater. It may be argued that such a receptor should not be described as a 5-HT receptor at all. In fact, this is not a real problem. By definition, the receptor should have a higher affinity for the presumed natural ligand than for other potential *endogenous* substances: thus, the 5-HT₂ receptor has extremely low affinity for dopamine or noradrenaline. It is of course impossible to test for all potential ligands, but it is very unlikely that a receptor for an endogenous amine will have appreciable affinity for any peptide transmitters or hormones. Secondly, the true discrepancy between K_d values for agonists and antagonists may be much smaller than it might appear.¹¹ Antagonists are generally much more lipophilic than the corresponding agonists. They therefore dissolve much more effectively in the lipid membrane environment around the receptor, and produce a very high local concentration of the compound, perhaps several hundred times higher than the nominal concentration in aqueous solution.

More careful analysis of binding data, using more sophisticated techniques, has led to increased complexities in interpretation. Small deflections in a displacement curve have been taken to indicate the

presence of distinct binding sites. This is often met with scepticism, but such assumptions may then be confirmed—or refuted—by other techniques. For instance, the brain 5-HT₁ receptor was divided into four subtypes (A, B, C and D) on the basis of binding data.^{12,13} The validity of this classification has been partially confirmed by information on second messenger systems and receptor structure. By contrast, the dopamine receptor was believed to have four subtypes.¹⁴ It is now generally recognized that there are probably only two, at least in the brain, but that each may exist in a high or a low affinity state with respect to various ligands.¹⁵ These problems raise the difficult and controversial problem of receptor classification: this will be discussed later in more detail.

There are other aspects to the use of radioligand binding methods. It is possible to link receptors and labelled ligands covalently, usually after solubilization of the receptors with non-denaturing detergents.¹⁶ It is then possible to identify the ligand–receptor complex by electrophoresis and detect it by autoradiography. This assists in receptor purification, assessment of receptor size and analysis of receptor sub-unit structure, if any. Autoradiography is the technique of using sensitive photographic film or emulsion to localize discrete areas of radioactivity, corresponding to the location of a receptor. The most important application of this method in receptor studies is in the detection of binding sites in histological sections.¹⁷ The practical procedures are very similar to those applicable to binding with membrane preparations, with the obvious difference that there is no filtration or centrifugation step. If possible, a radio-iodinated ligand is used, since it allows much shorter contact time between the labelled section and the photographic medium. Usually only hours or a few days are needed, while a tritiated compound may require several weeks. On the other hand, the resolution provided by a tritiated ligand is somewhat better.¹⁸

This type of autoradiography can be used both qualitatively and quantitatively. As an example of the former, we have shown that binding sites for [¹²⁵I]-neuropeptide Y (NPY) in the rabbit kidney are overwhelmingly located in the convoluted part of the proximal tubule, with very much binding in other sites.¹⁹ With the use of microdensitometry and computer-assisted image analysis it is also possible to estimate the density of binding sites in different locations, though probably with a greater margin of error than in conventional binding experiments.²⁰

Receptors and signal transduction

What happens when an agonist binds to its receptor? The answers to this have proved even more complex than had been suspected. In some cases it is possible to

give a relatively complete account of the intracellular events after binding, but this may prove illusory as some later examples will show. It is clear that there are many available mechanisms for signal transduction, but that some are very much more frequently used than others. Interestingly, some of these have proved to confirm ideas first proposed 30 or more years ago.

An agonist can interact with different cells by the use of different signalling mechanisms. For instance, there are vasopressin receptors on renal tubular cells and in vascular smooth muscle, using quite different signal transduction pathways. In the first instance, vasopressin activates adenylate cyclase,²¹ in the second it stimulates phosphoinositide turnover.²² These are the two most common signalling pathways in most cells and will be discussed further. Equally, most cells have a multiplicity of signalling mechanisms. A partial list, for vascular smooth muscle cells, is shown in Table II.

Table II Some endogenous agonists for the vascular smooth muscle cell. (Note that there is considerable heterogeneity in these cells, depending on vessel size, location and other factors)

<i>Contractile</i>
Vasopressin (V ₁)
Angiotensin II
Noradrenaline (α_1 , α_2)
Endothelin
5-hydroxytryptamine (5-HT ₂ , some 5-HT ₁)
Neuropeptide Y
Thromboxane A ₂
<i>Relaxant</i>
Noradrenaline (β_1 , β_2)
Dopamine (D ₁)
Calcitonin gene-related peptide
Endothelium-derived relaxant factor
Prostacyclin

The activation of adenylate cyclase, with the consequent increase in cellular cyclic adenosine 3',5'-monophosphate (cAMP) concentrations, was the earliest signal transduction mechanism to be delineated in non-excitabile cells²³ (Table III). Most of the subsequent events can be explained by the increased activity of a cAMP dependent protein kinase, which transfers phosphate groups from adenosine 5'-triphosphate to target proteins. Few of the latter have yet been identified.²⁴ As with many discoveries, the importance of cAMP was somewhat overestimated, and attempts were made to associate it with almost any receptor-mediated event: for instance, it was suggested that all dopamine receptors in the brain were positively linked to adenylate cyclase.²⁵ This was quickly refuted, and it was shown that some dopamine receptors were mirror-images, in that they caused the *inhibition* of adenylate cyclase²⁶ (Table III).

Table III Activators and inhibitors of adenylate cyclase. Receptor sub-type indicated where appropriate

<i>Activators</i>	
Noradrenaline (β_1, β_2)	
Dopamine (D_1)	
Histamine (H_2)	
Prostacyclin	
Calcitonin-gene related peptide	
5-hydroxytryptamine (some 5-HT ₁)	
Vasopressin (V_2)	
Vasoactive intestinal peptide	
<i>Inhibitors</i>	
Noradrenaline (α_2)	
Dopamine (D_2)	
Neuropeptide Y	
Opiates (δ, μ, κ)	
Adenosine (A_1)	

Shortly after this sequence of events another very significant observation was rediscovered.^{27,28} The cellular plasma membrane contains several types of phospholipids as well other lipids and an enormous variety of proteins. Most of these lipids are relatively stable, with slow rates of turnover. However, one small group, the phosphoinositides, are much more metabolically active. In the 1950s it was suggested that increased turnover of these lipids was related to cellular activation in certain cases. This was largely forgotten for over 20 years. It then re-emerged, with the added elaboration that increased phosphoinositide (PI) turnover was somehow related to intracellular calcium mobilization and calcium influx into the cell.^{29,30} The notion that calcium was an important intracellular regulator is even older, dating back to the early pharmacological studies of Loewi, Ringer and others on cardiac function. This, too, was a long-neglected area, though now it is recognized that the role of calcium may be even more important than that of cAMP in many cells. Work in this field has been enormously facilitated by development of methods for measuring calcium concentrations in living cells.³¹ Figure 2 is a simplified, schematic illustration of current concepts of PI metabolism and calcium homeostasis, while Table IV lists receptors known to be linked to stimulation of PI turnover.

Some parts of this scheme are very well established. There can be no doubt that inositol trisphosphate (IP3) is capable of causing calcium release from internal membrane stores.³² Equally, it is clear that calmodulin acts as an intracellular receptor, or acceptor, for calcium, and then acts as an activator for numerous other proteins.³³ One of the best known examples is the enzyme myosin light-chain kinase which is involved in smooth muscle contractility.³⁴ It is worth noting again that protein phosphorylation (and dephosphorylation) is a universal mechanism in

Figure 2 Greatly simplified scheme for agonist-stimulated phosphoinositide turnover, and related events. Gp: guanine nucleotide binding protein; PLC: phospholipase C; PI: polyphosphonositides; DAG: diacylglycerol; PKC: protein kinase C; IP3, IP4: inositol polyphosphates; PP: protein phosphorylation. Calcium is shown entering through a 'receptor-operated' channel.

regulating cell function at the molecular level.³⁵ Other issues are far from resolved. In particular, what is the role of the large number of other inositol phosphates which might be formed? Indeed, many of these have actually been detected in cells.³⁶ One possibility is that one or more may regulate calcium flux across the plasma membrane after receptor activation.³⁷ In some cells at least this seems to occur. It is also possible that receptor-mediated calcium influx—the so-called 'receptor-operated calcium channel'—largely serves to refill intracellular calcium stores which have been depleted.³⁸ Others believe that influx may precede calcium mobilization in some cells, such as the platelet.³⁹ Perhaps both views are valid, depending on cell and receptor type. There are also instances where an agonist may cause calcium influx via a receptor-activated channel without the involvement of PI metabolism.⁴⁰

Figure 2 mentions yet another kinase, protein kinase C (PKC).⁴¹ Realization of the importance of

Table IV Endogenous activators of phosphoinositide turnover. Receptor sub-type indicated where appropriate

Noradrenaline (α_1)
Vasopressin (V_1)
Histamine (H_1)
Angiotensin II
Bombesin
Endothelin
Acetylcholine (some muscarinic)
Thromboxane A2
Leukotriene D4

this enzyme is an even more recent development. It is involved in many vital cellular processes, such as proliferation and differentiation, pH homeostasis and secretion.⁴² It is now clear that there are several subtypes of PKC in different tissues, though the functional implications of this remain uncertain.⁴³ The activity of PKC is dependent on the presence of calcium and certain lipids, but it is thought to be further activated by diacylglycerols. These are produced by phospholipase C mainly from phosphoinositides, but also from other phospholipids such as phosphatidylcholine.⁴⁴ Other lipids, such as sphingosine, may be endogenous PKC inhibitors.⁴⁵ The interrelationship of PKC to the PI pathway is a complex one. It may have a synergistic role in some aspects of cell activation, for instance in the platelet.⁴⁶ On the other hand, PKC may exert a feed-back inhibitory effect on PI hydrolysis.⁴⁷ Highly potent activators are available, of which the tumour promoting phorbol ester is the best known. Inhibitors of the enzyme could have therapeutic potential in some circumstances, such as cancer chemotherapy, but existing agents do not combine high specificity and potency with low toxicity.⁴⁸

As mentioned previously, adenylate cyclase inhibition is also now recognized as an important signalling mechanism. It must be appreciated that cAMP is a bidirectional messenger: it may lead to activation or inhibition, whereas calcium is almost always activating. In the myocardial cell, for instance, increases in cAMP or calcium can increase contractility.⁴⁹ This is increasingly exploited therapeutically.⁵⁰ By contrast, calcium produces smooth muscle cell contraction while cAMP inhibits it.⁵¹ Agonists at smooth muscle α_2 -receptors cause contraction and inhibit adenylate cyclase, while similar but not identical receptors on the platelet mediate aggregation.⁵² In both instances there is a rise in intracellular calcium, but it is not clear whether or how this is related to changes in cAMP concentration. Merely reducing basal levels of the nucleotide does not seem to be an adequate stimulus for cell activation and increases in cell calcium. It has therefore been postulated that these receptors are also linked to other messenger systems, such as sodium-hydrogen exchange, possibly leading to a secondary influx of calcium.⁵³ This is controversial, but it is clear that this type of receptor is still poorly understood.

This is all, one would think, complex enough. It is nonetheless far from comprehensive. Another nucleotide, cyclic guanosine 3',5'-monophosphate (cGMP) is attracting increasing attention as a second messenger. The systems concerned are surprisingly different from those involving cAMP. The enzyme guanylate cyclase exists in two forms, one soluble and cytosolic, the other particulate and membrane-bound.⁵⁴ The former is a haemoprotein and is activated by organic nitrates and sodium nitroprusside. It is also stimulated by the

endothelium-derived relaxant factor (EDRF) first described by Furchgott.⁵⁵ Most researchers, though not all, believe that EDRF is nitric oxide, derived from the amino-acid arginine.⁵⁶ If this is so, EDRF is almost literally an 'endogenous nitrate'. There does not seem to be a membrane receptor, as it is usually understood, for this type of guanylate cyclase. There may, however, be intracellular receptor(s), possibly free thiol-containing compounds which can form nitrosothiol intermediates which, in turn, activate the enzyme:⁵⁷ this is still controversial.⁵⁸ The particulate guanylate cyclase is stimulated by the atrial natriuretic factors (ANFs).⁵⁹ The receptor and enzyme in fact form a very closely linked complex.⁶⁰ This enzyme is apparently not activated by the organic nitrates, though sodium nitroprusside is effective.⁶¹ As with cAMP, cGMP interacts with a specific nucleotide-dependent protein kinase.⁶² The principal effect is to reduce intracellular calcium concentrations by a variety of mechanisms. Increased calcium sequestration and extrusion, and decreased influx, all play a part in producing this outcome.⁶³ Understandably, therefore, agents that increase cGMP levels generally relax smooth muscle and inhibit platelet aggregation. Receptors negatively linked to either guanylate cyclase have yet to be discovered.

The versatility of the cyclic nucleotides has been further emphasized by the discovery of ion-channels directly controlled by cyclic nucleotide concentrations.⁶⁴ These channels appear to be of particular significance in olfactory and visual information transfer. Most of those so far discovered are regulated by cGMP.⁶⁵

These mechanisms account for most of the actions of classical neurotransmitters, and of many drugs and peptide hormones. However, many growth factors, including platelet-derived growth factor and insulin, act partly through yet another system. Their receptors incorporate a tyrosine kinase—the other kinases mentioned phosphorylate proteins at serine or threonine residues.⁶⁶ Again, the target substrates of this kinase are poorly defined, though they are known to include the receptor itself. The tyrosine kinase receptors are of particular interest in cancer research, because of their presumed involvement in the process of cell transformation and the fact that they may be encoded by cellular proto-oncogenes.⁶⁷

A few receptors cannot be classified under any of the above categories. The cytokine interleukin-1, for instance, appears to activate a novel second messenger system in which a phospholipase C is specifically linked to hydrolysis of phosphatidylethanolamine.⁶⁸ The detailed mechanism of action of other cytokines is even less clearly understood, and it is quite possible that other 'unorthodox' signalling pathways may be associated with these agents.

Throughout this discussion there has been rather

loose reference to the linkage of receptor and effector. How does this actually occur? Here too the details are beginning to emerge. In many, perhaps most, instances the crucial linking component is a guanine nucleotide binding (or G) protein.⁶⁹ These proteins bind and subsequently hydrolyse guanosine triphosphate. They were first described in the context of the regulation of adenylate cyclase and of retinal light receptors, but this class of proteins has since proliferated. Phospholipase C-linked receptors are linked through some form of G protein, though there appear to be several sub-types of G protein—for instance, there may be a G protein specific for the neutrophil.⁷⁰ The classification of G proteins, and investigation of their functions, has been aided by unexpected tools. Cholera toxin modifies and irreversibly activates the G protein (Gs) responsible for stimulating adenylate cyclase.⁷¹ Conversely, a component of pertussis toxin inactivates the G protein (Gi) which inhibits adenylate cyclase function.⁷² Pertussis toxin also inactivates some, but not all, of the G proteins linked to phospholipase C.⁷³ It remains to be seen whether specific agents become available for blocking pertussis toxin-insensitive G proteins. In any case, it is clear that potential roles for G proteins are constantly emerging. For instance, phospholipase A2 may be directly linked to receptors via a G protein, rather than being activated as a secondary result of increased intracellular calcium concentrations.⁷⁴ G proteins may also directly regulate membrane ion channels.⁷⁵

Receptors and molecular biology

Like all the other biomedical sciences pharmacology is increasingly influenced by the concepts and methods of molecular biology. The results are already very impressive. For many receptors the complete amino-acid sequence of the receptor protein has been established. This means that the nucleotide sequence of the gene is also known. For several receptors— α_1 and α_2 ,⁷⁶ β_1 and β_2 ,⁷⁷ 5-HT_{1A}, 5-HT_{1C}, 5-HT₂,⁷⁸ for instance—the genes have now been cloned. Further, in some instances the cloned gene has been introduced into cells which do not normally express it, and has been shown to retain biological activity. It is possible to identify regions of particular significance within a gene: parts of the gene that traverse the cell membrane, bind G proteins or bind external ligands. One result of this has been the discovery of surprising similarities in structure between apparently disparate receptors, such as β and 5-HT receptors.

The above genes are monomeric, consisting only of one polypeptide chain. Comparable progress has been made with multimeric receptors, made up of several dissimilar polypeptides. The nicotinic acetylcholine receptor, which is directly linked to an ion channel,

was the first example of this.⁷⁹ Another, of greater immediate therapeutic relevance, is the complex which constitutes the dihydropyridine sensitive, voltage-dependent calcium channel.⁸⁰ Here too the components have been identified and the binding sites for the different structural types of calcium antagonists have been localized. Thus, it can be shown that drugs such as nifedipine, verapamil, diltiazem and flunarizine all bind to distinct peptides. Interestingly, this finding was predicted from 'traditional' binding experiments, which showed allosteric interactions between the different ligands.⁸¹ There is therefore no obstacle to the characterization of the structure of even the most complex receptors, though it is clearly much more difficult to reconstruct an oligomeric structure *in vitro*.

Conclusions and prospects

It will be obvious that we know a great deal about 'receptive substances', though much of our knowledge is very recent. These are great intellectual and technical achievements, and their extent has perhaps not been widely appreciated. Many may wonder, nevertheless, if much of this will be of clinical relevance. Do we need to be concerned about the intricacies of receptor classification? Is the detailed molecular structure of a receptor of practical importance?

Receptor classification is certainly a cause of unease among pharmacologists. Even in receptors where classification was considered to be well established doubts have emerged. For instance, are there grounds for further sub-division of the α_1 receptor?⁸² Many of these difficulties are the results of success. Initially, a receptor was characterized by some measurable functional response, or its inhibition. Next, ligand binding techniques appeared, followed by analysis of signal transduction mechanisms. In many cases we now know the precise structure of the receptor. Systems of classification may be based on any of these properties, or on combinations of all of these. Occasionally there is general agreement on classification, as in the case of dopamine receptors in the brain. By contrast, there are several competing classifications of serotonin receptors, none of which seem fully adequate.^{83,84} As more information becomes available confusion often increases. Some pharmacologists and biochemists now suggest that the most satisfactory solution is the most radical one: to ignore virtually all information from the first three approaches and to base all taxonomy on receptor structure. As the previous section indicates, this can lead to unexpected relationships. Is this useful in practice? At the moment, not particularly. In the foreseeable future, though, this may change. As the work of Sir James Black has emphasized, random screening of vast numbers of newly-synthesized substances is not an efficient way to discover new drugs (it

also, incidentally, shows the practical relevance of accurate receptor classification—H₁ histamine antagonists are not very effective in treating peptic ulcers). If the three-dimensional structure of a receptor can be defined, it may be possible to design molecules to interact with precisely the appropriate site. The importance of such modelling techniques in determining quantitative structure–activity relationships is rapidly expanding. This may be of particular importance in designing agonists/antagonists for peptides.

Finally, the technology is now available to study some receptors directly *in vivo* in man. Ligands may be labelled with very short-lived positron emitting isotopes, and their binding to receptors can be assessed at least semi-quantitatively by positron emission tomography (PET).⁸⁵ Of course, this also provides an instantaneous picture of ligand distribution. One example of this technique has involved dopaminergic ligands in patients suffering from Parkinson's dis-

ease.⁸⁶ At the moment the availability of PET is very limited. The scanners are extremely expensive and a cyclotron is required virtually on-site to generate the isotopes. It is too early to say whether there will be any strong impetus to make this technique more widely accessible, either on diagnostic or on therapeutic grounds.

This brief review has concentrated on the basic aspects of recent receptor research. Even within those limits it has been very incomplete: for instance, nothing has been said of steroid receptors, which are not on the external cell membrane.⁸⁷ Nor have receptor synthesis, metabolism and internalization been discussed.⁸⁸ It is hoped that other articles will deal with these issues from a more clinical standpoint. Virtually no branch of medicine has remained unaffected, though all the implications have yet to be comprehended.

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