0031-6997/92/4403-0351$03.00/0
PHARMACOLOGICAL REVIEWS

Vol. 44, No. 3
Printed in U.S.A.

II. Definition of Pharmacological Receptors

TERRY P. KENAKIN,[1] RICHARD A. BOND,[2] AND TOM I. BONNER[3]

[1]*Division of Pharmacology, Glaxo Research Institute, Research Triangle Park, North Carolina;* [2]*Baylor College of Medicine, Center for Experimental Therapeutics, Houston, Texas; and* [3]*Laboratory of Cell Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland*

I. Introduction . . . 351
II. Receptors: an operational definition . . . 351
III. Functional tissue systems . . . 352
IV. Pharmacological criteria for classification . . . 352
A. Recognition . . . 352
1. Antagonists . . . 353
2. Agonists . . . 355
B. Transduction . . . 355
V. Molecular biology relevant to receptor characterization . . . 356
VI. Basis for a molecular nomenclature . . . 357
VII. Parallel pharmacological and molecular nomenclatures . . . 359
VIII. Suggested rules for naming receptors . . . 360
IX. Conclusions . . . 360
X. References . . . 361

I. Introduction

TRADITIONALLY, different receptors have been distinguished by drugs (chemicals) for which the receptors vary in their responsiveness. This has led to the gradual recognition of additional receptors for the same ligands. As the structures of the ligands have been refined, this has allowed the distinguishing of multiple subtypes of receptors. The central problem in identifying new receptors, thus far, has been in defining the significance of variations in pharmacological properties, i.e., what is the minimum difference that is necessary to establish a new subtype. The recent cloning of numerous receptor molecules for many of the major neurotransmitters has made it clear that there are often more clearly distinct receptor molecules expressed in a single mammalian species than can be easily distinguished with available drugs. Although these molecules can be expected to eventually provide the basis for identifying the corresponding receptors and may allow the development of more selective drugs, the current lack of sufficiently selective drugs means that it is difficult to establish the physiological functions that more selective drugs might target.

Nevertheless, it is clear that many more such receptors will be identified in the near future and that to minimize confusion in the literature, it will be necessary to arrive at a generally accepted definition of what constitutes new receptors, as well as a means of naming them. These issues have been central to the deliberations of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. In this paper, we summarize the current pharmacological and molecular approaches to the definition of the criteria by which alterations in cellular function resulting from the presence of a ligand can be ascribed to specific receptors for that ligand and present suggestions for the naming of these receptors.

II. Receptors: An Operational Definition

We will discuss some criteria by which alterations in cellular function resulting from the presence of a ligand can be ascribed to specific receptors for that ligand in the biological system. It will be axiomatic that the main criteria for qualification for the operational term receptor are the functions of *recognition* and *transduction*. By this definition, a receptor must recognize a distinct chemical entity and translate information from that entity into a form that the cell can read to alter its state accordingly, e.g., by a change in membrane permeability, activation of a guanine nucleotide regulatory protein, an alteration in the transcription of DNA.

Langley, a pioneer in the field of receptor research, defined a receptor (in his words, "receptive substance") as being the site of competition for agonists and antagonists (recognition) and the vehicle for the transmission of the stimulus of agonist interaction to the cell (transduction) for the production of a *physiological response*. The criterion of transduction may require further discussion for processes known to produce a biochemical change in a cell with an as yet unknown physiological

resultant. These criteria would exclude sites that are concerned with the biological disposition of molecules but not cellular metabolism (i.e., "silent" receptors). Included in this category would be the neuronal uptake site for catecholamines (targets for tricyclic antidepressants) and sites such as the clearance receptors for atrial natriuretic factor (Maack et al., 1987). For these to be included, modifications to the criteria of transduction would need to be made. This definition also distinguishes binding sites with no known physiological significance (i.e., "acceptors"; Furchgott, 1972; Ariens, 1984; Green and Maayani, 1987; Laduron, 1987) from receptors. This definition does not differentiate entities currently thought to be pharmacological receptors from enzymes. To differentiate these, we would stipulate that the recognition unit should not chemically alter the endogenous ligand. This would separate enzyme substrates from hormones, neurotransmitters, and other agonists for drug receptors.

III. Functional Tissue Systems

The relative merits, strengths, and weaknesses of different approaches to receptor pharmacology have been debated. In general, multicellular systems such as isolated tissues have the advantages resulting from stimulus amplification mechanisms, because these allow the quantification of agonist efficacy. Also, they are better predictors of drug response in humans. However, by their nature, these systems necessitate indirect approaches to receptors that binding does not require. A schematic of the relative merits of various systems used to study drugs is given in figure 1.

A relatively new development in this debate is the discovery that receptors may interact with each other on the level of the cell membrane by sharing effector couplers. For example, a common coupling G protein may be shared by opioid and α_2-adrenoceptors in NG108–15 cells (Lee et al., 1988), rabbit locus coeruleus neurons (Aghajanian and Wang, 1987; Christie et al., 1987), and rabbit cortex (Limberger et al., 1988). Under these circumstances, isolated tissues would be better predictors of organ responses to drugs than subcellular or reconstituted systems. However, from the point of view of receptor classification, these interactions may prove to be a serious complication.

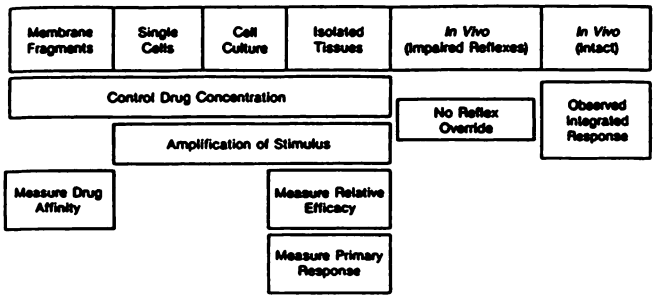

FIG. 1. Representation of different systems for the pharmacological study of drug receptors. Some theoretical advantages of various test systems (from Kenakin, 1987a).

Perhaps more relevant is the potential for isomerization of receptors in different systems (both physiological and artificial such as expression systems) by complex formation with membrane-bound proteins (i.e., G proteins). This is a well-known phenomenon for many agonists, making them unreliable tools for receptor classification. Ternary complex formation and receptor isomerization are *assumed* not to occur with antagonists. However, antagonism is a pharmacological definition bestowed upon a drug because it blocks the effects of an agonist and does not produce a measurable response. To completely rule out receptor isomerization, it must be shown that the experimental system possesses the reported properties and amplification characteristics to demonstrate low levels of agonism. In general, there is no reason a priori to assume that ternary complex formation among a ligand, receptor, and G protein will summarily promote dissociation of the α, β, and γ subunits of the G protein and, thus, reveal agonism. Recent data concerning antagonists that actively destabilize ternary complex formation (Costa and Herz, 1989; Costa et al., 1992) show that the observed affinity can be greatly dependent on G proteins in membrane systems. Under these circumstances, the environment of the receptor can determine measured affinity, thereby introducing theoretical and practical problems with the use of such antagonist affinity data for receptor classification. These ideas are discussed further in relation to the concepts of negative efficacy and receptor precoupling (vide infra).

IV. Pharmacological Criteria for Classification

A prerequisite for a meaningful discussion of possible criteria for receptor definition should be the presentation of the limitations of the methods used to estimate the parameters on which the criteria are based.

A. Recognition

Discussion of the definition of receptors inevitably centers on the criteria for classification of receptors. Much has been written about the pharmacological criteria for receptors, both from the point of view of binding and isolated tissue studies (Furchgott, 1972; Black, 1981; Molinoff et al., 1981; Laduron, 1984; Laduron, 1987; Green and Maayani, 1987; Kenakin, 1987a; Nahorski, 1987; Braestrup and Andersen, 1989; Green, 1990). In terms of receptor binding studies, it is generally agreed that ligands must have selectivity, bind in a saturable manner, and, where appropriate, demonstrate stereoselectivity for the proposed receptor. It is expected that there be a class of chemicals that bind selectively to receptors, in a saturable manner, that can be displaced competitively by other chemicals or removed by washing with drug-free media. Often added to this list is the need for an endogenous agonist in addition to selective binding with foreign ligands. This would exclude classifications

such as cannabinoid receptors for which, currently, there is no known endogenous agonist. In such cases, this condition has less to do with definition of the receptor than with its proper identification. Although the principle of naming a receptor after its endogenous agonist should be maintained wherever possible, the withholding of receptor status from an entity, which otherwise satisfies all criteria for defining a receptor, could be counterproductive. Such "receptors" should be accepted as receptors with the understanding that the name should be changed when the identity of the endogenous ligand becomes clear. One potential error in doing so is that we might be giving two names to the same receptor, if its endogenous ligand turns out to be one for receptors that already have been identified. A related error, e.g., the identification of subtypes of substance P receptors before the identification of the related peptides, neurokinin A and neurokinin B, is that we incorrectly give one agonist name to a group of receptors that actually has a group of closely related endogenous agonists. Occasional errors of both types are unavoidable in a useful nomenclature system.

The traditional method of receptor classification has been by the relative potencies and selectivities of antagonists and agonists. Specifically, the following criteria have been used: (*a*) affinity of antagonists, (*b*) potency ratio of agonists, (*c*) affinity of agonists, and (*d*) relative intrinsic efficacy of agonists. It is worth considering these methods and how they are used in the receptor classification process.

1. Antagonists. The measurement of antagonist potency in functional assays by the Schild technique (Arunlakshana and Schild, 1959) has yielded an abundance of data for receptor classification. Under these conditions, the abscissal intercept of a Schild regression (under equilibrium conditions with compounds interacting at a single site) with the antagonist tested over a concentration range yields an important parameter for receptor classification. If the regression is linear with unit slope, this parameter is defined as an estimate of the equilibrium dissociation constant of the antagonist-receptor complex. If one assumes that antagonists bind competitively to the agonist-binding site, differences in the equilibrium dissociation constants of antagonist-receptor complexes measured with this method can be used to define receptor subtypes. A suggested standard for the proposal of a novel receptor subtype is for the dissociation constant (K_B) of a compound (or ideally two compounds with chemically diverse structures) to exhibit a 1 log unit or greater difference from its value(s) at known receptors. In general, the minimal requirements for accurate estimation of K_B values by this method are as follows.

1. Measurements must be made under equilibrium conditions (Furchgott, 1972, 1978). There is much data to show how nonequilibrium conditions can give the *appearance* of equilibria and simple competitive antagonism (Kenakin, 1987b) yet yield erroneous estimates for K_B (fig. 2). Three major objectives in establishing equilibrium conditions are: (*a*) elimination of sites of loss including biological processes such as uptake systems and degradative enzymes, as well as protection from physicochemical breakdown (i.e., autooxidation of catechols) of the ligands interacting with the receptor; (*b*) the elimination of the possible interference from endogenously released ligands that interact with the receptor; and (*c*) the achievement of temporal equilibrium. In general, these conditions are of much greater concern for agonists than antagonists, particularly when using isolated tissue preparations.

2. The Schild regression is based on the assumption that the observed antagonism is of the simple competitive type. Under these conditions, a slope that is different from unity has no meaning in terms of the chemical constant of interaction between an antagonist and a receptor. Therefore, if the slope is not statistically different from unity, it should be constrained to unity (MacKay, 1978) and the intercept utilized as an estimate of the K_B. This is based on the assumption that an infinite number of assays would yield a slope exactly equal to unity.

3. The regression should be linear with unit slope over a considerable concentration range (minimally 30- to 100-fold where possible). There are instances in which physiological antagonism (e.g., β-adrenoceptor-mediated inhibition of cholinergic contraction in guinea pig trachea; Kenakin, 1982) can produce linear Schild regressions of unit slope. This usually cannot be demonstrated over large ranges of concentration.

4. One possible criterion for defining receptor differences via Schild analysis is a difference in the elevation of regression lines, i.e., a difference in location along the

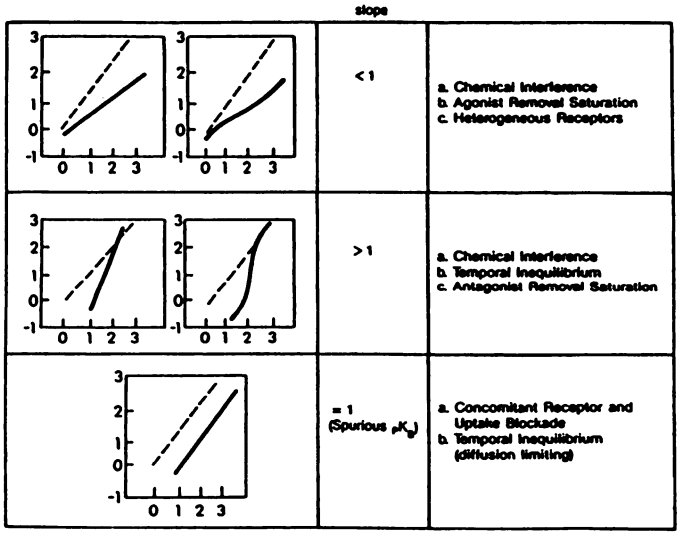

FIG. 2. Potential Schild regressions and possible experimental conditions that would cause deviation from simple competitive behaviour (from Kenakin, 1987b).

log antagonist axis that would indicate a difference in K_B (Snedecor and Cochran, 1967). Because regression lines are used to characterize antagonist potency, a statistical comparison would remove subjectivity (Kenakin and Black, 1978). This also would eliminate characterizations such as "receptor subtype X-like" when two regressions are similar but not coincident.

A potential complicating factor in Schild analysis is the case in which receptors are substantially precoupled to G proteins in the absence of agonists (Costa et al., 1990, 1992). Under these circumstances, there would be a heterogeneity of binding sites available to the antagonist, if the precoupled receptor has a different conformation than the uncoupled one. This also raises the specter of negative efficacy for antagonists that may stabilize the guanosine diphosphate-G protein complex. The potency of such antagonists then would be subject to the relative proportions of receptor and G protein and, thus, would be tissue dependent (Wregget and DeLean, 1984; Costa and Herz, 1989; Costa et al., 1992). The degree of precoupling of receptors can produce a sinistral shift of the Schild regression but no detectable nonlinearity (Costa et al., 1992). Therefore, no obvious indication of coupling protein effects would be seen by Schild analysis. Such drugs would vary in effectiveness in different systems of varying degrees of receptor precoupling, which, in turn, has been shown to vary with experimental conditions such as cellular integrity (Costa et al., 1992). Presently, it is unclear to what extent this is a significant hazard in classification of receptors with antagonists, but differences in observed antagonist affinity with cellular integrity and/or cyclic nucleotide levels would be indicators of a potential problem.

Although a case could be made for a purely pharmacological definition of receptors based on the quantitative scale of potency of antagonists, a point to be considered is the possibility that different *binding domains* of receptors then might be classified as unique receptors. It is known from deletion mutation studies that different binding domains for agonists and antagonists can be differentiated for agonists and antagonists. These data and that of others open the possibility that many drugs thought to be competitive with agonists for common binding sites may, in fact, be allosteric effectors binding to sites physically removed from the agonist-binding site.

A possibly useful technique to differentiate true competitiveness at a common site on the receptor and allosteric interaction is *resultant analysis* with which the antagonism of a "test" antagonist is measured in conjunction with that of a "reference" antagonist (Black et al., 1986; Kenakin and Beek, 1987; Leff and Morse, 1987). This method is similar to the additive dose ratio method to determine competitiveness (Paton and Rang, 1965) but has the added advantage of compensating for secondary effects of the test antagonist. This is because the "control" dose-response curve is determined in the presence of the test antagonist, thereby canceling secondary effects on the blockade produced by the reference antagonist thereafter. Resultant analysis can be used to differentiate apparent simple competitive antagonism from allosteric interaction (Kenakin and Boselli, 1989).

There are data to suggest that the agonist binding for at least some G protein-linked receptors is contained within the seven membrane-spanning portions of the receptors and that these regions are the most highly conserved in terms of amino acid homology across species and tissues. If allosteric effectors are used for receptor classification and these drugs bind to the heterogeneous outer portions of receptors removed from the endogenous agonist-binding site, then it would be possible that heterogeneous binding across species and tissues could result not from differences in the endogenous agonist-binding domain but, rather, from differences in allosteric modulation sites. Under these circumstances, it would be possible that receptor heterogeneity would not be detected by the interaction of the receptor with endogenous agonists but only with foreign ligands. Thus, it could be conceived that the classification of receptors on the basis of allosteric sites might open a "Pandora's box" of heterogeneity because, conceivably, receptors constructed in various cells from different species would be different, due to the availability of different materials for receptor construction. In practical terms, such a Pandora's box might translate into a cornucopia of potential pharmacological selectivity. However, the danger for therapeutic pharmacology would be that structure-activity relationships, based on such regions in one test system used for screening molecules for biological activity, might not have a counterpart in the human receptor.

There now is mounting evidence that, even when all known nonequilibrium conditions are effectively neutralized or allowed for, there still is considerable heterogeneity in estimated K_B values as measured in a functional system. One daunting prospect is the possibility of variable affinity of ligands for receptors resulting from differences in membrane components (Bevan et al., 1989). It is well known that differential purification of receptors can produce differences in binding profiles for agonists and antagonists. Of potentially greater concern are data to suggest that *flexibility* is a characteristic of receptors required for their function with respect to transduction. Under these circumstances, the rearrangement of subunit structure would be a major determinant of ligand affinity and this, in turn, would be sensitive to the environment for that receptor (Weinstein, 1987). This protean nature of receptors suggests that uniform classification, based solely upon ligand recognition, would be more variable than assumed, if the receptor were simply a recognition unit with no transducing properties. The potential for negative efficacy and receptor precoupling should introduce caution in assum-

ing that ligand affinity for receptors will provide the gold standard for classification.

2. Agonists. Receptors also have been classified pharmacologically with agonists, but the possible problems with this approach are compounded by the added drug property of intrinsic efficacy. Specifically, agonists possess the properties of affinity (like antagonists) and also *efficacy,* the ability to impart a change of state in the receptor. Historically, agonist potency ratios have been the established method of receptor classification by agonists, the most well-known example being the classification of adrenoceptors into α and β subtypes by Ahlquist (1948). The premise is that the potency of an agonist depends upon the drug factors of intrinsic efficacy and affinity and, also, the tissue factors of receptor density and the efficiency with which the receptors are coupled to effector mechanisms. In a given tissue in which two agonists are compared directly, the tissue factors cancel and their relative potency depends only upon the drug parameters efficacy and affinity. Under these circumstances, the *relative* potency of agonists becomes a receptor "fingerprint" which can be utilized in receptor classification. Sometimes, this method is degraded to the use of the relative order of potency for classification, but this can lead to serious errors. Given the prerequisite that the relative potencies of agonists depend only upon drug-related parameters, agonist potency ratios should be useful measures of receptor subtypes. However, recent evidence indicates that the magnitude of both the affinity and efficacy of agonists may not be independent of tissue factors.

In terms of the affinity of agonists for receptors, even when care is taken to attain equilibrium conditions in isolated tissue preparations, theoretical modeling and experimental data have shown that the scales of potency and selectivity of agonists all can be obfuscated in intact systems (MacKay, 1987; Kenakin, 1989). If the agonist induces a change in the receptor to which it is binding (e.g., as in an ion channel from a closed to an open state, the formation of a ternary complex with a membrane-coupling protein), then the existing methods for the estimation of the affinity of agonists for receptors yield an *apparent* affinity that may characterize the complete mechanism of receptor activation (Colquhoun, 1987). This would include membrane components in addition to the receptor, and, therefore, these data would not be useful for receptor classification (MacKay, 1978; Leff and Harper, 1989; Leff et al., 1990; MacKay, 1990; Kenakin et al., 1990). Errors in the estimation of agonist affinity would lead to concomitant errors in agonist potency ratios.

B. Transduction

By definition, the *transduction* aspect of receptor mechanisms suggests selective interaction of the receptor with unique membrane or cytosolic components of the cell that then carry the message imparted by the drug. Theoretically, part of the receptor classification process could include information about the transducing recognition sites on the receptor and even to the overall observed physiological response. In terms of agonist activity, there are two possibilities for receptor classification: the *type* or the *magnitude* of the response.

The type of response clearly is inadequate as a tool for classification. Data from a number of sources demonstrate considerable "cross-talk" between receptors and effector systems. It has been shown in reconstitution studies that many receptors are capable of interacting with more than one type of G protein, and there is suggestive evidence that this may occur physiologically in cells (Kenakin, 1988, 1990). Classification by physiological function is not practical from the point of view that a given biochemical process may have different effects in different cells depending on the processing of the biochemical signal. Also, some agonists are known to be pleiotropic, producing many biochemical effects in cells that may also be differentially coupled to cytosolic processes. Therefore, it is conceivable that agonists of low intrinsic efficacy would activate only the most efficiently coupled of these processes and produce a profile of activity different from that of a powerful high-efficacy agonist. Under these conditions, agonists of differing intrinsic efficacy could produce different pharmacological effects by activating the *same* receptor. For example, opioid receptors mediate the inhibition of adenylate cyclase and the stimulation of GTPase in NG108–15 cells, but the latter effect is more sensitive to diminution of receptor density (smaller effective receptor reserve) (Costa et al., 1988). Presumably, the stimulation of GTPase also would be less sensitive to activation by low-efficacy agonists. Given these constraints, classification by effector would seem to be unsuitable.

The other possibility is to use the magnitude of response to a given agonist for classification. It is clear that the intrinsic activity of agonists is not a useful parameter for receptor typing, because its magnitude varies with the efficiency of stimulus-response coupling. There are numerous examples of low-efficacy agonists that produce direct responses only in highly efficiently coupled tissues and act only as antagonists in other systems. Therefore, null methods must be used to factor out the tissue effects and, hopefully, yield parameters relating only to the agonist-receptor pairs. Theoretically, the measurement of relative efficacy of agonists fulfills this function, but again, the complexity of membrane dynamics needs to be considered. Measurements of efficacy and relative potency would be invalid measures of receptor selectivity in cases in which receptors are promiscuous with respect to the effector coupling proteins with which they interact (Kenakin, 1988; Kenakin and Morgan, 1989). For example, in IMR-32 tumor cells, the M_3 muscarinic acetylcholine receptor subtype selectively

couples to phosphoinositide hydrolysis and the M_4 subtype to inhibition of adenylate cyclase, but in cells containing M_2 subtype mRNA, the expressed M_2 receptor couples to both responses (Pinkas-Kramaski et al., 1990). An example of where receptor promiscuity, with respect to receptor-effector coupling mechanisms, may occur in native membranes is in rat pancreatic acinar cells. In this system, cholecystokinin functionally interacts with six G_s proteins and with G_i1, G_i2, and G_i3 proteins as well (Schnefel et al., 1990). Another may be adenosine A_1 receptors from bovine brain which copurify with G_i1, G_i2, and G_o when activated by an agonist (Munshi et al., 1991). The ability of this receptor to interact with all three G proteins with agonist activation was confirmed in reconstitution studies. Under these circumstances, different agonists could predispose the formation of different ternary complexes, making the relative availability of G proteins in various membranes a determinant of efficacy. In these systems, the relative agonist potency and/or intrinsic efficacy would not be useful scales for receptor classification.

Given these potential pitfalls to the use of agonists for receptor classification, the following would be a minimal list of requirements for their use. (*a*) As for antagonists, equilibrium conditions should be attained. For agonists, this may be more difficult in view of biochemical mechanisms in tissues that are designed to remove endogenous agonists from the receptor compartment (i.e., degradative enzymes, uptake processes). It is a prerequisite for receptor classification studies that the concentration of drug at the receptor be known accurately. (*b*) Potency ratios should be used and not rank order of potency. The correct use of agonist potency ratios always ensures correct rank orders, but the converse is not always true. (*c*) If a receptor subtype is being classified, the endogenous agonist for that receptor subtype must be included in the analysis. (*d*) Agonists should demonstrate stereoselectivity at the receptor where appropriate.

V. Molecular Biology Relevant to Receptor Characterization

Two problems have plagued a purely pharmacological definition of receptor subtypes. The first is the question of whether observed pharmacological differences represent different receptor molecules or simply cell-specific differences in the environment of a common receptor molecule. This question has arisen in several forms in the preceding discussion. The second is the problem of knowing whether there is a pure receptor population in a given tissue, a problem that may generally have been underestimated. The cloning of receptors has revealed that there are often more different receptor molecules for a given ligand than generally recognized pharmacological subtypes. Although one might be inclined to expect that, therefore, there will be a distinct receptor molecule everywhere there is a reasonable suggestion of a pharmacological subtype, one should expect that in some cases there will be fewer distinct molecules than there are apparent pharmacological subtypes, because of cell or species-specific variations in the properties of what should be considered a single receptor molecule (see section IV for a discussion of species variation). A corollary of the existence of previously unrecognized subtypes is the observation that the occurrence of multiple subtypes in a tissue, or even a cell line, is more common than had been generally recognized. The introduction of molecular biology in the form of cloned receptors provides a basis for alleviating these problems.

The characterization of cloned receptors should eventually provide the information on which to base a nomenclature that truly reflects the molecular identity of the receptors so that a particular subtype of receptor will refer to a specific protein (or set of proteins for multiple subunit receptors). In particular, the expression of cloned receptors in cell lines will provide pure populations of receptors in identical cellular environments so that the factors that allow the different receptors to be operationally distinguished can be identified. To provide a pure population of receptors, the untransfected host cell line into which the cloned receptors are introduced must not produce measurable amounts of any of the receptor subtypes in question. Although it is relatively easy to find such cell lines, it is not yet clear how many or what specific cell lines should be used to have an adequate representation of the cellular environments that the receptor experiences in vivo. For example, the fibroblast cell lines which molecular biologists have used for initial characterization of G protein-coupled receptors may not contain significant amounts of specific G proteins that interact with the receptors in their natural cellular environment. Because we do not know with which specific G proteins individual receptors interact or even what the full repertoire of G proteins is, the choice of the most appropriate host cell lines is not immediately obvious. However, some pharmacological properties of these receptors, such as antagonist affinities, may not depend on which G proteins are present so that the choice of host cell line will not be critical.

The cloned receptors also provide new methods for determining which receptor molecules are present in a given cell or tissue. The DNA sequences of the clones can provide probes of high subtype specificity for the detection of receptor mRNAs by a variety of methods, such as northern blots and in situ hybridization. These methods vary in the sensitivity for detecting low levels of mRNA, as well as in their spatial resolution. For example, in situ hybridization is capable of identifying individual cells in tissue sections, whereas northern blots typically use RNA extracted from a whole tissue. Thus, if there is a low level of mRNA expressed in many cells of a given tissue, a northern blot that sums the signal from all of the cells will be more sensitive than in situ

hybridization, whereas in situ hybridization will be more sensitive if there are only a few cells expressing more substantial levels of mRNA. Other methods such as solution hybridization coupled with probe protection assays or reverse transcription coupled with polymerase chain reaction amplification offer potentially greater sensitivity, although generally not with the single-cell resolution of in situ hybridization. Unfortunately, the relative amounts of two mRNAs may not reflect the relative amounts of receptor protein in a given tissue for two reasons. First, there can be large differences in the efficiency with which different messages are converted into protein. Second, at least for tissues containing neuronal cells, in which the mRNA is located in the cell body but the receptors are located predominantly on axonal and dendritic projections, there may be a substantial difference between the location of the mRNA and the receptor protein. Although not yet widely available, subtype-specific antibodies against portions of the cloned receptor sequences should allow more accurate determination of receptor distributions and heterogeneity. For example, antibodies specific for the different muscarinic acetylcholine receptors have been made using as antigens proteins derived from expression in *Escherichia coli* of most of the domain connecting the fifth and sixth transmembrane domains (Levey et al., 1991).

All of the receptors that have been cloned and sequenced belong to one of a small number of families of structurally similar proteins, such as the ligand-gated ion channels, which have several different but related subunits, and the G protein-coupled receptors, which are single-subunit receptors characteristically having seven membrane-spanning domains. For some ligands, such as acetylcholine, there are receptors, nicotinic and muscarinic, that belong to more than one structural family. Other examples are $GABA_A$* versus $GABA_B$ and 5-HT_3 versus 5-HT_1 and 5-HT_2. The different structures generally correspond to distinctly different modes of signal transduction and can be used as a primary means of subdividing receptors for a given ligand. There is, however, a second structural class of receptors that can interact with G proteins. These are the receptors for peptides such as insulin and insulin-like growth factors (Okamoto et al., 1990). They consist of two identical subunits, each of which has only a single membrane-spanning domain.

VI. Basis for a Molecular Nomenclature

The most important contribution of molecular biology to receptor characterization is that it allows the identification of the actual molecules that underlie the pharmacology and provides an evolutional perspective with which to identify species-specific variation in receptor pharmacology, which is essential for extrapolating results in animal models to human therapeutics.

* Abbreviations: GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine.

The major questions concerning a molecular definition of receptor subtypes are how different does the sequence of a molecule have to be to justify a new name, and how can one establish the molecular identity of the receptor that mediates a physiological response. If we identify the receptors by their protein sequences, which seems to be the most appropriate molecular identifier, we should attach the same name to all minor variants in sequence such as naturally occurring alleles or in vitro created mutants. If such variants differ in any important receptor properties, then they would be referred to as specific variants, e.g., the Ala197 allele or mutant of a given receptor. Differences in glycosylation could be referred to as different glycosylation states if glycosylation proves to be important. However, evidence based on mutating the glycosylation sites of the β_2-adrenoceptor (Rands et al., 1990) and the m_2 muscarinic acetylcholine receptor (van Koppen and Nathanson, 1990) indicates that lack of any or all glycosylation sites has little effect on the pharmacology. Likewise, variants resulting from alternative splicing, as in the case of the dopamine D_2 receptor, should be considered as length variants of a single-receptor subtype. The two D_2 sequences differ by the insertion or deletion of 29 amino acids near the middle of the cytoplasmic domain connecting the fifth and sixth transmembrane domains. Because this domain has been implicated in the specificity of G protein binding for other G protein-linked receptors, it has been suggested that the two forms may bind to different G proteins (Eidne et al., 1989; Giros et al., 1989). If this were to be the case, variant names should be used to distinguish the two molecules. However, the sequence difference occurs in the portion of the cytoplasmic domain that can be deleted from both adrenoceptors and muscarinic acetylcholine receptors without affecting functional response (Strader et al., 1987; Shapiro and Nathanson, 1989). Thus, the biological significance of this difference remains to be determined. On the other hand, biologically significant alternative splicing does occur in the glutamate receptor genes Glu-A, -B, -C, and -D (Sommer et al., 1990). The alternatively spliced forms of these receptors, which provide different sequences for a 38-amino acid region preceding the fourth transmembrane domain, have been referred to as the "flip" and "flop" forms. Although these may not be the most suitable names for these alternative forms of the proteins, it is clear that they should be given different names because they impart different channel properties.

More substantial sequence variations, in the approximate range of 1 to 10%, such as those that occur between mammalian species during the course of evolution of receptor molecules, also should be included under a single name. This assumes that it is clear that the molecules are true homologs, i.e., the sole descendants of the same molecule in the most recent common ancestor of the two species being considered. The rationale for doing so is

that function is generally conserved during evolution so that subtypes having the same name could be expected to have the same physiological functions in different species. The evolution of protein sequences for families of related proteins, such as the hemoglobins, has been the subject of study for more than 20 years and provides the foundation for identifying which sequences are true homologs. If there are the same number of functional genes for different subtypes of a given receptor in the two species, it is generally not difficult to identify which pairs of receptors, one from each species, are the true homologs. Thus, assuming that all of the subtype genes existed in the ancestor of all mammals, one can expect that the sequences for a single subtype in all mammals will be more closely related to each other than the various subtypes in any one species are to each other. However, if there have been gene duplications since the evolutionary divergence of the two species and if the duplicated gene has acquired a function, then the correspondence may not be clear because there are now two genes derived from the ancestral gene in one species but only one in the other species. One can expect that the two genes in the first species will be more similar to each other than they are to the single gene in the second species. The question of gene duplication will have to be resolved for each gene family, i.e., each set of receptors for the same ligand and having the same general receptor structure. In general, this complication can be expected to be relatively infrequent among mammals but more serious when comparing mammalian receptors to receptors from other vertebrates or even invertebrates.

In principle, if one has, for example, a rat receptor sequence and wants to know whether it is the homolog of one of the known human subtype sequences, one would need the sequences of all the human subtypes for that ligand to determine with certainty which was the most closely related to the rat sequence. In practice, one probably would have a potentially incomplete set of human subtype sequences to compare and, therefore, would want to know whether the rat sequence is the homolog of one of the known sequences or represents a new subtype. If the rat sequence is not significantly more closely related to one of the human sequences than to the others, it almost certainly represents a new subtype. If it differs by 1 to 10% from one of the sequences, it is probably the homolog of that sequence. For G protein-coupled receptors for which the same receptor has been cloned from several species such as cow, pig, rat, mouse, hamster, and human, the homologous genes typically have 85 to 95% amino acid sequence identity. The membrane-spanning domains of these receptors are quite conserved between subtypes, but there are regions (the amino terminal to the beginning of the first transmembrane domain, between the fifth and sixth transmembrane domains, and following the seventh transmembrane to the carboxyl terminal) that show little conservation not only in sequence but in their length. Examination of such regions can often be more useful than the overall sequence when getting an indication of whether two sequences are species homologs. When comparing sequences, one should be alert to the possibility of sequencing errors. The most troublesome error is a frame-shift error, which causes incorrect translation of an extensive portion the DNA sequence into amino acid sequence when a single nucleotide is erroneously deleted or inserted. Such errors are not uncommon. If there is a second compensating error that results in restoration of the correct reading frame, the errors are often not uncovered until sequences are compared between different laboratories or between species (Guyer et al., 1990).

In some cases, it may be difficult to decide whether two sequences are species homologs without supplementary information. The most useful information is provided by a DNA hybridization test, whereby one determines what human gene is most closely related by hybridizing a probe derived from the rat sequence to a Southern blot of restriction enzyme digests of human genomic DNA and asking whether the most stable (with respect to temperature) bands of hybridization correspond to those characteristic of one of the known human genes. This test requires that the probes used to define the characteristic patterns of the known human genes represent as closely as possible the same region of the amino acid sequence as the rat probe so that the homologous rat and human probes would detect precisely the same restriction fragments. Just as one should use several drugs to characterize a receptor, one should use several restriction enzymes for such an analysis to avoid the possibility that two different genes might give the same restriction fragments for a single randomly chosen enzyme. Another form of corroborating evidence would be whether the receptor (either protein or mRNA) has the same tissue distribution in the two species, assuming, of course, that the distributions are sufficiently distinct to distinguish among subtypes.

Because the receptors have not evolved to distinguish among various synthetic ligands and changing even a single amino acid in the receptor can cause substantial changes in ligand affinities (Suryanarayana et al., 1991), there is reason to expect that there will be species differences for some synthetic ligands (even though the affinity for the endogenous ligand may be unchanged) for what we would identify as a single molecular subtype. Such differences will need to be noted in the pharmacological definitions attached to the molecular names and are clearly of major importance in selecting animal models for testing drugs intended for human use. The 5-HT_{1B} and 5-HT_{1D} receptors provide a clear case of such species variation. Even before the recent cloning of these receptors, it appeared plausible that they might represent such species differences of a single molecule based on their similar distributions and functional properties but

apparent mutual exclusion in different species. From the cloning of these receptors, it is now clear that there are two receptors in humans with 5-HT_{1D} pharmacological properties, whereas the rat and mouse homologs of one of these two genes has 5-HT_{1B} pharmacological properties (Hartig et al., 1992). The advantage of identifying such species variants as the same receptor is that function can be expected to be largely conserved between species, even if there are changes in structure that alter its interaction with synthetic ligands.

Individual subtypes of multiple-subunit receptors can be molecularly defined by identifying each of the subunits that are present, e.g., the R1 receptor could be defined as containing the a1, b3, c2 and d5 subunits. A major unresolved question is which of the multitude of possible combinations of subunits actually occurs to form in vivo receptors.

The question of identifying which molecule mediates a particular physiological response will largely depend on having discriminating drugs available, but in some cases, such as cell lines or tissue samples, molecular tools such as specific DNA probes for hybridization to mRNA or subtype-specific antibodies generated from clone-derived antigens may be helpful in identifying which subtypes are present or absent. However, absence is always difficult to establish and mRNA distribution in tissue may not coincide with the receptor distribution. It may also be possible to identify the physiological function of specific subtypes by purely genetic means, such as gene inactivation or replacement in transgenic animals or the inhibition of translation of specific mRNAs by the use of antisense RNAs. To the extent that there are known molecular subtypes for which discriminating drugs have not yet been identified, caution would dictate that the receptor be identified as, for example, "molecule1 or possibly molecule3." Attention should be given to the possibility that more than one receptor type is involved which necessitates using more than one or two drugs to make the identification.

VII. Parallel Pharmacological and Molecular Nomenclatures

The cloned receptors that are presently available have not yet been studied thoroughly enough to allow a full definition for any set of receptors that would allow the unambiguous assignment of a molecular name to an in vivo receptor, especially in the case in which multiple related receptors may be present. Nevertheless, the characterization of cloned adrenoceptors and muscarinic acetylcholine receptors has advanced far enough that we can predict with a reasonable amount of confidence the basic factors that will be important in defining subtypes of G protein-coupled receptors. Similarly, the cloning of a substantial number of subunits of nicotinic acetylcholine receptors and $GABA_A$ receptors has defined the problems but has not yet provided the solutions for defining molecular subtypes of ligand-gated ion channel receptors. The task of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification is to find a uniform framework for defining receptor names that will allow all receptors to be defined according to the same principles so that the subcommittees devoted to particular receptor types can formulate nomenclature proposals with a common format. To minimize changes in the nomenclature and in the associated definitions with the advent of new information, especially new subtypes, we should try to predict which factors are essential to the definition of subtypes and which factors are sufficiently variable (e.g., with cellular environment) that they should not be considered as parts of the primary definition. The latter factors might or might not be included as secondary information in much the same way as a dictionary frequently includes information distinguishing the usage of words with similar meanings. To the extent that one cannot accurately predict all the factors, a framework must be designed that allows the incorporation of new information into the definitions without drastically altering the nomenclature. However, substantial changes in definition while retaining the same names may generate some confusion.

Given our present state of knowledge, we need a transitional system such as the format suggested by J. P. Green (1990), or as used in the *Trends in Pharmacological Science* nomenclature supplement (vol. 12, 1991), which can incorporate most of the observationally relevant information to provide parallel pharmacological and structural (or molecular) definitions. Such a format allows the correspondence between these two definitions to be indicated. However, to avoid misinterpretation, there should be an explicit assessment of the reliability of the correspondence between molecular and pharmacological subtypes. One should not indicate that a particular molecular subtype corresponds to a given pharmacological subtype simply because its pharmacological profile most closely matches that of the pharmacological subtype, when, in fact, several molecular subtypes may not be distinguished by the pharmacological definition. One would hope that, as additional data become available concerning the pharmacological properties of cloned receptors such that the pharmacological definitions can be refined, the information that does not allow unambiguous distinction of the molecular identities will be moved to footnote status, and the pharmacological and molecular definitions will coalesce. There is general agreement that receptors should be defined as a ligand-binding site with a ligand-mediated functional response, the response being biochemical, electrical, or mechanical in its manifestation. There is also general agreement that the primary label in the names should be the endogenous ligand (or, provisionally, another ligand when the endogenous ligand is unknown). Whether the compendium of receptors should list the ligands alphabetically or alphabeti-

cally within chemical types (such as amines, amino acids, peptides, etc.) is open for discussion. They could easily be listed one way with an index listing them the other way. A secondary classification, which is well grounded in molecular structure and is observationally distinguishable, would be on the basis of structural type of receptor, e.g., G protein-coupled or ligand-gated ion channel. Such a distinction has already been made in some cases, such as nicotinic versus muscarinic acetylcholine receptors or $GABA_A$ versus $GABA_B$ receptors, but not in other cases, such as the 5-HT_3 receptor which is not given a name distinctly different from the 5-HT_1, 5-HT_2, and 5-HT_4 receptors. This is one subgrouping of receptors for the same ligand that is not likely to change with additional information and, therefore, could safely and usefully be incorporated into the nomenclature.

VIII. Suggested Rules for Naming Receptors

We are faced with the twin problems of receptor nomenclature and classification. To the extent that they can be distinguished, nomenclature implies a set of names with definitions that allows a one-to-one correspondence between names and objects (such as receptors) with specific properties, whereas classification implies a hierarchical ordering of a group of objects based on their degree of similarity to each other. There is clearly a pressing need for an agreed upon set of names and a set of criteria for defining what constitutes a new receptor subtype, but it is not clear that there is a major need for these names to reflect an extensive classification scheme. The existing tendency toward classification in receptor nomenclature is a natural consequence of the process in which new subtypes generally have been identified through the use of new drugs, which could distinguish different receptors within a previously unresolved subtype. In contrast, we can expect that in the future many new subtypes will be identified as distinct molecules through cloning and, in many cases, the drugs capable of distinguishing the subtypes will have to be developed subsequently. Given our relatively incomplete knowledge of the number of receptors and of the full range of the properties of those receptors that have already been identified, it would probably be a mistake in terms of stability of the nomenclature to incorporate more than the most rudimentary classification, i.e., the ligand and the structural type (G protein-coupled, ligand-gated ion channel, etc.), into the nomenclature.

Classification neutral labels such as 1, 2, 3, . . . are preferred to labels such as 1A, 1B, 2A, 2B, . . ., which imply differing degrees of similarity, because the perceived relationships between subtypes can change with the advent of new subtypes or new drugs. It is inevitable that such changes will occur if the relationships are based on a few properties of the receptors. This is clearly the case in the taxonomy of plants and animals in which, if one considers only a few specific characteristics, one can derive different phylogenetic trees depending on which characteristics are used. The case of the 5-HT_{1C} receptor is evidence that the same thing can easily happen with receptors. It was originally classified as a 5-HT_1 subtype based on a single drug but subsequently appeared to be more similar to 5-HT_2 both pharmacologically and by comparison of the cloned sequences. If relationships are not implied by the names, the relatedness of different subtypes could be included as supplementary information so that it can be modified without modifying the nomenclature. As a rule, the more subtypes there are, the more difficult it will be to define meaningful relationships. Clearly, there will be cases, such as adrenoceptors, in which a nomenclature that is not classification neutral is so well established that it would not make sense to change the whole system unless it were to become unwieldy because of the identification of even more subtypes.

Although it was expedient for the α_2-adrenoceptors, the naming of molecular subtypes after their chromosomal location is not desirable. Generally, the human chromosomal location is not established for some time after a cloned receptor is published, especially if it is not a human clone. Furthermore, there may be more than one subtype on the same chromosome as in the case of the m_1 and m_4 muscarinic acetylcholine receptors.

IX. Conclusions

For the present, two ongoing systems of receptor definition, structural and pharmacological, appear to be necessary. One would be based upon receptor structure with potentially incomplete pharmacological criteria. After pharmacological criteria have been fulfilled, these receptors would be defined in both systems. The other would define receptors by pharmacological criteria (i.e., endogenous agonist, antagonists). Again, it would be conceivable that there would be incomplete data for some receptors defined in this category, therefore, precluding definition in the other. A "completely" defined receptor would possess a unique pharmacological profile based on agonist and antagonist data, a known endogenous ligand, and a distinct amino acid sequence. It may be that receptor identification by structure will prove to be a technically more simple task than identification by ligand pharmacology if receptor environment (i.e., lipid, coupling proteins) plays a significant role in defining the pharmacology of some receptors. In these cases, receptor pharmacology would vary with the tissue or cellular system but not be indicative of different receptor types. The resolution of the question of whether receptor environment is a significant factor in receptor identification and/or classification will require the use of systems in which the structural identity of the receptor is well defined.

The nomenclature system will clearly evolve at a rate proportional to the diligence of all concerned in recon-

ciling the structural and pharmacological definitions. As an initial framework for implementing these parallel sets of definitions in a form that facilitates their reconciliation, the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification has adopted a tabular format closely resembling the *Trends in Pharmacological Sciences* receptor nomenclature supplement.

Acknowledgments. We acknowledge the in-depth comments concerning this paper by Dr. Don Jenkinson, University College, London, United Kingdom.

REFERENCES

AGHAJANIAN, G. K., AND WANG, Y-Y.: Common α_2- and opiate effector mechanisms in the locus coeruleus: intracellular studies in brain slices. Neuropharmacology **26:** 793–799, 1987.

AHLQUIST, R.: A study of the adrenotropic receptors. Am. J. Physiol. **153:** 586–600, 1948.

ARIENS, E. J.: Receptors; perspectives in pathology and clinical medicine. J. Recept. Res. **4:** 1–7, 1984.

ARUNLAKSHANA, O., AND SCHILD, H. O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. **14:** 48–58, 1959.

BEVAN J. A., BEVAN, R. D., AND SHREEVE, S. M.: Variable receptor affinity hypothesis. FASEB J. **3:** 1696–1704, 1989.

BLACK, J. W.: Receptors in the future. Postgrad. Med. J. **57** (suppl. 1): 110–112, 1981.

BLACK, J. W., GERSKOWITCH, V. P., LEFF, P., AND SHANKLEY, N. P.: Analysis of competitive antagonism when this property occurs as part of a pharmacological resultant. Br. J. Pharmacol. **89:** 547–555, 1986.

BRAESTRUP, C., AND ANDERSEN, P. H.: Receptor subtypes and endogenous ligands; rational tools in the search for psychotropic drugs? *In* Clinical Pharmacology in Psychiatry, ed. by S. G. Dahl and L. F. Gram, pp. 3–9, Springer-Verlag, Heidelberg, 1989.

CHRISTIE, M. J., WILLIAMS, J. T., AND NORTH, R. A.: Cellular mechanisms of opioid tolerance: studies in single brain neurons. Mol. Pharmacol. **32:** 633–638, 1987.

COLQUHOUN, D.: Affinity, efficacy and receptor classification: is the classical theory still useful? *In* Perspectives on Receptor Classification: Receptor Biochemistry and Methodology, vol. 6, ed. by J. W. Black, D. H. Jenkinson, and V. P. Gerskowitch, pp. 41–49, Alan R. Liss, New York, 1987.

COSTA, T., AND HERZ, A.: Antagonists with negative intrinsic activity at δ opioid receptors coupled to GTP-binding proteins. Proc. Natl. Acad. Sci. U.S.A. **86:** 7321–7325, 1989.

COSTA, T., KLINZ, F.-J., VACHON, L., AND HERZ, A.: Opioid receptors are coupled tightly to G proteins but loosely to adenylate cyclase in NG108–15 cell membranes. Mol. Pharmacol. **34:** 744–754, 1988.

COSTA, T., LANG, J., GLESS, C., AND HERZ, A.: Spontaneous association between opioid receptors and GTP-binding regulatory proteins in native membranes; specific regulation by antagonists and sodium ions. Mol. Pharmacol. **37:** 383–394, 1990.

COSTA, T., OGINO, Y., MUNSON, P. J., ONARAN, H. O., AND RODBARD, D.: Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. Mol. Pharmacol. **41:** 549–560, 1992.

EIDNE, K. A., TAYLOR, P. L., ZABAVNIK, J., SAUNDERS, P. T. K., AND INGLIS, J. D.: D2 receptor, a missing exon. Nature **342:** 865, 1989.

FURCHGOTT, R. F.: The classification of adrenoreceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. *In* Handbook of Experimental Pharmacology, Catecholamines, vol. 33, ed. by H. Blaschko and E. Muscholl, pp. 283–335, Springer-Verlag, New York, 1972.

FURCHGOTT, R. F.: Pharmacological characterization of receptors. Its relation to radioligand-binding studies. Fed. Proc. **37:** 115–120, 1978.

GIROS, B., SOKOLOFF, P., MATRES, M.-P., RIOU, J.-F., EMORINE, L. J., AND SCHWARTZ, J.-C.: Alternative splicing directs the expression of two D2 dopamine receptor isoforms. Nature **342:** 923–926, 1989.

GREEN, J. P.: Pharmacological receptors: the need for a compendium of classification, nomenclature and structure. Trends Pharmacol. Sci. **11:** 13–16, 1990.

GREEN, J. P., AND MAAYANI, S.: Nomenclature, classification, and notation of receptors: 5-hydroxytryptamine receptors and binding sites as examples. *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol. 6, ed. by J. W. Black, D. H. Jenkinson, and V. P. Gerskowitch, pp. 237–267, Alan R. Liss, New York, 1987.

GUYER, C. A., HORSTMAN, D. A., WILSON, A. L., CLARK, J. D., CRAGOE, E. J., JR., AND LIMBIRD, L. E.: Cloning, sequencing and expression of the gene encoding the porcine α_2-adrenergic receptor. J. Biol. Chem. **265:** 17307–17317, 1990.

HARTIG, P. A., BRANACHEK, T. A., AND WEINSHANK, R. L.: A subfamily of 5-HT_{1D} receptor genes. Trends Pharmacol. Sci. **13:** 152–159, 1992.

KENAKIN, T. P.: The schild regression in the process of receptor classification. Can. J. Physiol. Pharmacol. **60:** 249–265, 1982.

KENAKIN, T. P.: The Pharmacologic Analysis of Drug Receptor Interaction, Raven Press, New York, 1987a.

KENAKIN, T. P.: What can we learn from models of complex drug antagonism in classifying hormone receptors? *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol. 6, ed. by J. W. Black, D. H. Jenkinson, and V. P. Gerskowitch, pp. 169–185, Alan R. Liss, New York, 1987b.

KENAKIN, T. P.: Are receptors promiscuous? Intrinsic efficacy as a transduction phenomenon. Life Sci. **43:** 1095–1101, 1988.

KENAKIN, T. P.: Challenges for receptor theory as a tool for drug and drug receptor classification. Trends Pharmacol. Sci. **10:** 18–22, 1989.

KENAKIN, T. P.: Drugs and receptors; the state of the art. Drugs **40:** 666–687, 1990.

KENAKIN, T. P., AND BEEK, D.: Measurement of antagonist affinity for purine receptors of drugs producing concomitant phosphodiesterase blockade: the use of pharmacologic resultant analysis. J. Pharmacol. Exp. Ther. **243:** 482–486, 1987.

KENAKIN, T. P., AND BLACK, J. W.: The pharmacological classification of practolol and chloropractolol. Mol. Pharmacol. **14:** 607–623, 1978.

KENAKIN, T. P., AND BOSELLI, C.: Pharmacologic discrimination between receptor heterogeneity and allosteric interaction: resultant analysis of gallamine and pirenzepine antagonism of muscarinic responses in rat trachea. J. Pharmacol. Exp. Ther. **250:** 944–952, 1989.

KENAKIN, T. P., AND MORGAN, P. H.: Theoretical effects of single and multiple transducer receptor coupling proteins on estimates of the relative potency of agonists. Mol. Pharmacol. **35:** 214–222, 1989.

KENAKIN, T. P., MORGAN, P. H., AND LUTZ, M.: Drug and tissue factors both confound K_A measurements. Trends Pharmacol. Sci. **11:** 273–274, 1990.

LADURON, P. M.: Criteria for receptor sites in binding studies. Biochem. Pharmacol. **33:** 833–839, 1984.

LADURON, P. M.: Limitations of binding studies for receptor classification. *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol 6, ed. by J. W. Black, D. H. Jenkinson, and V. P. Gerskowitch, pp. 71–79, Alan R. Liss, New York, 1987.

LEE, S., ROSENBERG, C. R., AND MUSACCHIO, J.: Cross-dependence to opioid and α_2-adrenergic receptor agonists in NG108–15 cells. FASEB J. **2:** 52–55, 1988.

LEFF, P., DOUGALL, I. G., HARPER, D. H., AND DAINTY I. A.: Errors in agonist affinity estimation: do they and should they occur in isolated tissue experiments? Trends Pharmacol. Sci. **11:** 64–67, 1990.

LEFF, P., AND HARPER, D. H.: Do pharmacological methods for the quantification of agonists work when the ternary complex mechanism operates? J. Theor. Biol. **140:** 381–397, 1989.

LEFF, P., AND MORSE, J. M.: Resultant pharmacological actions of verapamil: quantification of competitive 5-hydroxytryptamine antagonism in combination with calcium antagonist. J. Pharmacol. Exp. Ther. **240:** 284–287, 1987.

LEVEY, A. I., KITT, C. A., SIMONDS, W. F., PRICE, D. L., AND BRANN, M. R.: Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J. Neurosci. **11:** 3218–3226, 1991.

LIMBERGER, N., SPATH, L., AND STARKE, K.: Presynaptic alpha$_2$ adrenoceptor, opioid kappa-receptor and adenosine A_1-receptor interactions on noradrenaline release in rabbit brain cortex. Naunyn Schmiedebergs Arch. Pharmakol. **338:** 53–61, 1988.

MAACK, T., SUZUKI, M., ALMEIDA, F. A., NUSSENZVEIG, D., SCARBOROUGH, R. M., MCENROE, G. A., AND LEWICKI, J. A.: Physiological role of silent receptors of atrial natriuretic factor. Science **238:** 675–678, 1987.

MACKAY, D.: How should values of pA_2 and affinity constants for pharmacological competitive antagonists be estimated? J. Pharm. Pharmacol. **30:** 312–313, 1978.

MACKAY, D.: Use of null equations, based on classical and ternary models of drug action to classify receptors and receptor-effector systems even if some of the agonists and antagonists available have additional actions on the tissues. *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol. 6, ed. by J. W. Black, D. H. Jenkinson, and V. P. Gerskowitch, pp. 193–206, Alan R. Liss, New York, 1987.

MACKAY, D.: Agonist potency and apparent affinity: interpretation using classical and steady-state ternary-complex models. Trends Pharmacol. Sci. **11:** 17–22, 1990.

MOLINOFF, P. B., WOLFE, B. B., AND WEILAND, G. A.: Quantitative analysis of drug-receptor interactions: II. Determination of the properties of receptor subtypes. Life Sci. **29:** 427–443, 1981.

MUNSHI, R., PANG, I.-H., STERNWEIS, P. C., AND LINDEN, J.: A_1 adenosine receptors of bovine brain couple to guanine nucleotide-binding proteins G_{i1}, G_{i2}, and G_o. J. Biol. Chem. **266:** 22285–22289, 1991.

NAHORSKI, S. R.: What are the criteria in ligand binding studies for use in receptor classification? *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol. 6, ed. by J. W. Black, D. H. Jenkinson, and W. P. Gerskowitch, pp. 51–59, Alan R. Liss, New York, 1987.

OKAMOTO, T., KATADA, T., MURAYAMA, V., UI, M., OGATA, E., AND NISHIMOTO, I.: A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. Cell **62:** 709–711, 1990.

PATON, W. D. M., AND RANG, H. P.: The uptake of atropine and related drugs

by intestinal smooth muscle of the guinea pig in relation to acetylcholine receptors. Proc. R. Soc. Lond. B. **163:**1–44, 1965.

PINKAS-KRAMASKI, R., EDELMAN, R., AND STEIN, R.: Indications for selective coupling to phosphoinositide hydrolysis or to adenylate cyclase inhibition by endogenous muscarinic receptor subtypes M3 and M4 but not by M2 in tumor cell lines. Neurosci. Lett. **108:** 335–340, 1990.

RANDS, E., CANDELORE, M. R., CHEUNG, A. H., WILL, W. S., STRADER, C. D., AND DIXON, R. A. F.: Mutational analysis of the β-adrenergic receptor glycosylation. J. Biol. Chem. **265:** 10759–10764, 1990.

SCHNEFEL, S., PROFROCK, A., HINSCH, K.-D., AND SCHULZ, I.: Cholecystokinin activates G_i1-, G_i2, G_i3- and several G_s-proteins in rat pancreatic acinar cells. Biochem. J. **269:** 483–488, 1990.

SHAPIRO, R. A., AND NATHANSON, N. M.: Deletion analysis of the mouse M1 muscarinic acetylcholine receptor: effects on phosphoinositide metabolism and down-regulation. Biochemistry **28:** 8946–8950, 1989.

SNEDECOR, G. W., AND COCHRAN, W. G.: Statistical Methods, Iowa State University Press, Ames, IA, 1967.

SOMMER, B., KAINANEN, K., VERDOORN, T. A., WISDEN, W., BURNASHEV, N., HERB, A., KOHLER, M., TAKAGI, T., SAKMANN, B., AND SEEBURG, P. H.: Flip and flop: a cell-specific functional switch on glutamate-operated channels of the CNS. Science **246:** 1580–1585, 1990.

STRADER, C. D., DIXON, R. A. F., CHEUNG, A. H., CANDELORE, M. R., BLAKE, A. D., AND SIGAL, I. S.: Mutations that uncouple the β-adrenergic receptor from Gs and increase agonist affinity. J. Biol. Chem. **262:** 16439–16443, 1987.

SURANARAYANA, S., DAUNT, D. A., VON ZASTROW, M., AND KOBILKA, B. K.: A point mutation in the seventh hydrophobic domain of the α_2 adrenergic receptor increases its affinity for a family of β receptor antagonists. J. Biol. Chem. **266:** 15488–15492, 1991.

VAN KOPPEN, C. J., AND NATHANSON, N. M.: Site-directed mutagenesis of the M2 muscarinic acetylcholine receptor: analysis of the role of N-glycosylation in receptor expression and function. J. Biol. Chem. **265:** 20887–20892, 1990.

WEINSTEIN, H.: Classification based on ligand binding: on the chemical meaning of ligand affinity in studies of drug receptor interactions. *In* Perspectives on Receptor Classification, Receptor Biochemistry and Methodology, vol. 6, ed. by J.W. Black, D.H. Jenkinson, and V.P. Gerskowitch, pp. 41–49, Alan R. Liss, New York, 1987.

WREGGET, K. A., AND DE LEAN, A.: The ternary complex model: its properties and application to ligand interactions with the D_2-dopamine receptor of the anterior pituitary gland. Mol. Pharmacol. **26:** 214–227, 1984.