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RADIOLIGAND BINDING STUDIES OF ADRENERGIC RECEPTORS: New Insights into Molecular and Physiological Regulation[1]

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INTRODUCTION

The advent of direct radioligand binding studies has altered the entire complexion of the experimental approach to the study of adrenergic receptors. The first successful direct binding studies of β-adrenergic receptors were reported in 1974 (1–3) and those of α-adrenergic receptors only in 1976 (4–6). Nonetheless, the application of these methods over the past several years has led to rapid progress in a number of areas. In this article we review some of the highlights of this progress. It has not been our goal to be encyclopedic or all-encompassing. Rather, we have deliberately selected several areas for more extensive coverage. These choices have been, obviously, subjective ones. In general we have focused on studies that provide new insights into factors that appear to regulate the function of the adrenergic receptors, either at a physiological or biochemical level. Only material related to α- and β-adrenergic receptors published through the end of 1978 has been systematically considered.

Several radioligand antagonists have been widely used to study the β-adrenergic receptors in a variety of tissue systems. These are (–) [^{3}H]

[1]Abbreviations: [^{3}H]DHA, (–)[^{3}H]dihydroalprenolol; [^{3}H]DHE, [^{3}H]dihydroergocryptine; [^{3}H]HBI, (±)[^{3}H]hydrobenzylisoproterenol; IHYP, (±)[^{125}I]iodohydroxybenzylpindolol.

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dihydroalprenolol (7), (±) propranolol (8), and (±) [^{125}I] iodohydroxylbenzylpindolol (9). In addition to the radiolabeled β-adrenergic antagonists, the agonists (±) [^{3}H] hydroxybenzylisoproterenol (10, 11) and (±) [^{3}H] isoproterenol (12) have also been used to study the β-receptors. A variety of ligands have been used to study the α-adrenergic receptors. These include the antagonists [^{3}H] dihydroergocryptine ([^{3}H]DHE) (4,5) and [^{3}H] WB4101 (6, 13) and the agonists [^{3}H] clonidine, [^{3}H] epinephrine, and [^{3}H] norepinephrine (6, 13, 14). The multiplicity of ligands available for studying α-adrenergic receptors has led to a variety of different binding patterns, the precise explanation for which has not been entirely clear from published studies. Nonetheless, there is compelling evidence that when used under appropriate experimental conditions, each of the ligands described above does in fact label physiologically relevant α-adrenergic receptor binding sites. The apparent explanation for the different ligand binding patterns (which are dealt with in some detail below) appears to relate to heterogeneity of α-adrenergic receptors and the varying regulatory properties of these receptors.

Another major new insight about the receptors that has come from direct binding studies has been the growing appreciation of the very dynamic nature of their regulation by a variety of hormonal and other influences. Thus, the original conception of receptors as rather static entities in cell membranes that function mainly as input receivers for the hormonal or drug signal is being revised to that of a much more dynamic entity. It has been learned that not only the catecholamines themselves but a variety of hormones such as thyroid hormones, progesterone and cortisone, can all strikingly alter the numbers and the properties of the adrenergic receptors, thereby influencing cellular responsiveness to the catecholamines. The molecular mechanisms through which these varied regulatory phenomena are mediated, however, remain in most cases to be worked out.

α-ADRENERGIC RECEPTOR SUBTYPES

Ahlquist made the initial demarcation between α- and β-adrenergic receptors in 1948 (15). Subsequently, in 1967, Lands et al (16) provided evidence that there exist two subtypes of β receptors, namely, β_1 and β_2. β_1 and β_2 receptors are operationally defined by their affinities for epinephrine and norepinephrine; β_1 receptors have approximately equal affinity for epinephrine and norepinephrine whereas β_2 receptors have a higher affinity for epinephrine than norepinephrine (generally at least tenfold). More recently it has been recognized, in pharmacological experiments carried out in a variety of tissues, that there also exist at least two subtypes of α receptors.

These pharmacological studies have been extensively reviewed elsewhere (17–21).

There has been no uniformity in the terminology used to describe these subtypes. The α receptors involved in the feedback inhibition of norepinephrine release from nerve terminals have been called presynaptic α receptors or α_2 receptors (22); the α receptors typically found on effector cells have been called postsynaptic α receptors or α_1 receptors (22). We favor the terminology of "α_1" and "α_2" receptors proposed by Berthelson & Pettinger for a variety of reasons. First, the anatomical definition suffers from the weakness that in most systems studied the "presynaptic" α receptors (α_2 receptors) have not actually been demonstrated to occur on the nerve terminals themselves. Indeed, in some tissues there is reason to believe that these receptors may indeed not be physically located on nerve terminals since their number is not reduced by denervation of the tissue (23, 24). In rat heart, however, the total number of [^{3}H]DHE binding sites has been reported to decrease after denervation with 6 OH-dopamine (25). This could reflect loss of α_2 receptors on nerve terminals but so far this has not been actually demonstrated. Second, the anatomical definition would tend to preclude the existence of so-called presynaptic α receptors on other than presynaptic sites. However, it has been demonstrated by radioligand binding studies that human platelets (26) have such α receptors; hence it is preferable, in our view, to refer to these α receptors as α_2. Indeed there are probably other examples of postsynaptic α_2 receptors mediating many functions, such as, inhibition of renin release from the juxtaglomerular cells (22).

It is our purpose here to discuss the application of radioligand binding techniques to the issue of α-adrenergic receptor subtypes. These α receptor subtypes have been demarcated by their different affinities for a host of agonist and antagonist compounds (17–22, 26–28). Thus far, no simple, generally applicable definition of α_1 and α_2 receptors in terms of a potency series of agonist drugs has emerged analogous to that for the β receptor subtypes. Antagonists may serve to be more useful in separating α receptor subtypes. As determined by radioligand binding techniques, prazosin has been found to be ~ 10,000-fold α_1 selective (26–28) and yohimbine ~ 500-fold α_2 selective (26). Selectivity of these compounds is best determined by constructing competition curves using the α antagonist [^{3}H] dihydroergocryptine which does not discriminate between α_1 and α_2 receptors. Similarly, nonselective antagonists such as phentolamine (26) yield steep monophasic competitive curves whereas selective compounds yield flatter biphasic curves. The proportion of α_1 and α_2 receptors as well as the affinity constants of a selective drug for each site may be determined from competition curves by utilizing recently described computer modeling techniques (26, 29, 30).

Alternatively putatively selective radiolabeled drugs can be used to label α_1 or α_2 receptors. Snyder and his collaborators have identified heterogeneous mixtures of α receptors in brain membranes (6, 13, 14). Originally these investigators considered their data as supporting the notion of discrete "agonist" and "antagonist" states of the α receptor. They noted that agonists competed with higher affinity than antagonists at [^{3}H]clonidine, [^{3}H]epinephrine, or [^{3}H]norepinephrine sites whereas antagonists competed with higher affinity than agonists at sites labeled with the antagonist [^{3}H]WB-4101. Indeed, Exton and collaborators (30a) have adopted this conceptual framework of "agonist-antagonist" states to rationalize their binding data for the agonists [^{3}H]epinephrine and [^{3}H]norepinephrine and the antagonist [^{3}H]dihydroergocryptine in liver membranes. However, Snyder and his collaborators have discarded this hypothesis, no longer believing that α receptors exist in "agonist" or "antagonist" states; they have identified the agonist state as being α_2 receptors and the antagonist state as being α_1 receptors (23, 28, 31). In these studies the antagonist [^{3}H]WB-4101 was used to label α_1 receptors and the agonist [^{3}H]clonidine to label α_2 receptors. It is not yet clear whether the apparent α_1 selectivity of [^{3}H]WB-4101 in brain membranes will apply in other tissues to the extent that exclusively α_1 receptors and no α_2 receptors are labeled by this drug. Similarly, caution is indicated in using [^{3}H]agonists such as clonidine, epinephrine, or norepinephrine in efforts to measure α_2 receptors since it may be that only the α_{2_H} state (see following section) is being labeled by the low concentrations of the [^{3}H]agonists used in most assays. For these reasons, at the present time, it is our view that the most secure way to quantitate α-adrenergic receptor subtypes is by constructing competition curves using the nonselective radioligand [^{3}H]DHE and selective α antagonists such as prazosin and yohimbine and then analyzing the data with available computer modeling techniques. Indeed, a similar approach is available for measuring mixtures of β_1 and β_2 receptors using the nonselective β antagonists IHYP or [^{3}H]dihydroalprenolol and selective β antagonists (19, 32); these methods represent an improvement over previously available graphical techniques (33).

This recently developed methodology for measuring α_1 and α_2 receptors in tissues that contain complex mixtures of these receptor subtypes should open fruitful areas of investigation. For example, it has long been known that the pharmacological responsiveness and innervation of the rabbit uterus is sensitive to modification by sex hormones; indeed changes in α receptor numbers have been noted under different hormonal conditions (34, 35). It will now be possible to measure changes in the α receptor subtypes under these conditions; this could be particularly interesting since each subtype might be regulated independently.

REGULATION OF β- AND α-ADRENERGIC RECEPTORS BY GUANINE NUCLEOTIDES AND IONS

Most, if not all, β-adrenergic effects are mediated by stimulation of the enzyme adenylate cyclase. By contrast, it appears that many effects of α-adrenergic receptors are not mediated through an interaction with the adenylate cyclase system. However, certain α effects, often those mediated by so called α_2-adrenergic receptors (see above) do appear to be mediated by an inhibition of the activity of the enzyme adenylate cyclase (36, 39). Despite the opposite directional influences of the α- and β-adrenergic receptors on adenylate cyclase activity, strikingly similar patterns have emerged from studies of the interaction of these two receptor types with the adenylate cyclase system (40).

Major new insights have developed from studies which have focused in parallel on ligand binding and adenylate cyclase enzyme assays. Perhaps the most fundamental of these observations has concerned the role of guanine nucleotides in coupling receptor occupancy by agonists with activation or inhibition of adenylate cyclase catalytic activity. These observations are based on the original observations of Rodbell and colleagues almost a decade ago (41) that guanine nucleotides such as GTP are required for glucagon activation of hepatic membrane adenylate cyclase. It has subsequently been demonstrated that stimulation of adenylate cyclase through β receptors (11) or its inhibition through α-adrenergic receptors (42) requires the presence of guanine nucleotides such as GTP or nonhydrolyzable guanine nucleotide analogues such as Gpp(NH)p. However, in the absence of guanine nucleotides, enzyme stimulation by activators such as fluoride ion can still be observed. In parallel with these studies on adenylate cyclase activity it was observed several years ago that guanine nucleotides exert important agonist-specific regulatory effects on the β-adrenergic receptors (43, 44). More recently, very comparable findings have been observed in an α-adrenergic receptor system (40). These observations relate to the fundamental properties of agonist and antagonist interaction with the α- and β-adrenergic receptors as manifest in ligand binding experiments. Figures 1 and 2 show typical experimental data which highlight the agonist-specific effects of guanine nucleotides on β-adrenergic receptor binding in a model system, the frog erythrocyte membrane. Using (–)[^{3}H]dihydroalprenolol as the radioligand, it can be observed that the competition curve of an antagonist such as alprenolol is steep (slope factor[2] $\simeq$ 1) and uniphasic (46). Nonlinear least squares computer modeling techniques have revealed that such data are best interpreted as representing the interaction of alprenolol

[2]A slope factor is a measure of the steepness of a competition curve (45).

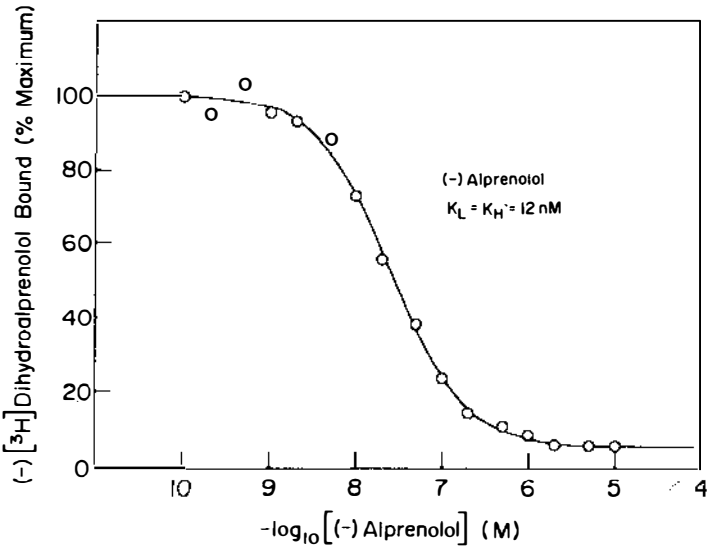

Figure 1 Computer modeled curves for inhibition of (–)[^{3}H]DHA binding to frog erythrocyte membranes β-adrenergic receptors by (–)alprenolol. Experiments were performed as described elsewhere using purified frog erythrocyte membranes and (–)[^{3}H]DHA at a concentration of ~ 2 nM. Data points represent means of duplicate determinations from a representative experiment. The curve is a theoretical one drawn by computer modeling procedures for a model with a single binding affinity state for (–)alprenolol of 12 nM. Attempts to fit these binding data to a two-state model did not improve the fit. K_L = low affinity dissociation constant. K_H = high affinity dissociation constant. These data are taken from ref. (46).

with a single binding affinity state of the receptor. Entirely comparable results have been obtained with other antagonists in similar experiments. As indicated in Figures 1 and 2, the computer-derived affinity constant of alprenolol for the receptors is 12 nM (24).

As shown in Figure 2 the situation observed with agonists is quite different. In the absence of added guanine nucleotide the competition curve of isoproterenol (a full agonist) is shallow with a slope factor significantly less than 1.0 (~0.7). When computer modeling of these data is performed it is found that the experimentally determined data points are statistically much better fit ($p < .01$) to a two-state binding model than to a one affinity state model. We have designated the affinities of isoproterenol for these two states K_H (the affinity for the higher affinity sites) and K_L (the affinity for the lower affinity sites) respectively. As noted in Figure 2, there is about a 60-fold difference in affinities of isoproterenol for the high and low affinity states. Approximately 75% of the receptors are found in the high affinity state whereas about 25% are seen to be in the lower affinity state. In the

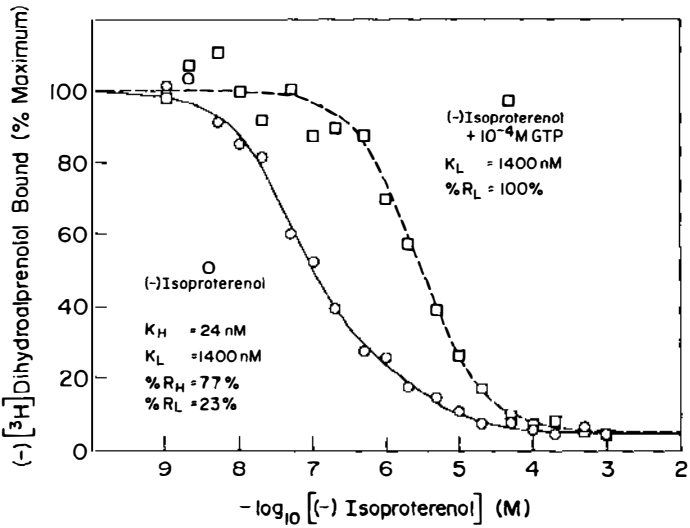

Figure 2 Computer modeled curves for inhibition of (–)[^{3}H]DHA binding to frog erythrocyte membrane β-adrenergic receptors by (–)isoproterenol in the presence and absence of GTP. Procedures as described in legend to Figure 1. Data points are means of duplicate determinations from a representative experiment. The curve for isoproterenol alone was significantly better fitted ($p < 0.01$) by a two-state binding model, whereas in the presence of GTP a one-state model was adequate to fit the data points. K_H, K_L as defined in legend to Figure 1. $\%\ R_H = \%$ receptors in high affinity state, $\%\ R_L = \%$ receptors in low affinity state. When both curves were fitted together and constrained to share the value for K_L, there was no significant worsening of the fitted curves compared to the results obtained when each curve was fitted separately. Data taken from (46).

presence of saturating concentrations of guanine nucleotides, such as Gpp(NH)p the competition curve is shifted to the right and now displays a slope factor of ~ 1. Computer modeling of this curve now shows only a single homogeneous class of binding sites with an affinity not discernibly different from that of the lower of the two affinities observed in the absence of guanine nucleotides. Thus, guanine nucleotides appear to mediate a transition between the high and low affinity states of the receptor which are observed in the presence of agonists. Not surprisingly, the guanine nucleotides have no effect on the competition curve of antagonists which appear to be associated with a homogeneous state of binding affinity. Entirely analogous findings have been observed with [^{3}H]DHE binding in human platelet membranes. Thus, competition curves by antagonists such as phentolamine are steep and model to one site (26). Competition curves of full agonists such as norepinephrine are best fit to two affinity states in the absence of guanine nucleotides and to a single binding state of homogeneous

low affinity in the presence of guanine nucleotides (B. B. Hoffman, D. Mullikin-Kilpatrick, and R. J. Lefkowitz,; submitted for publication).

It has also been observed in both of these systems that a divalent cation such as magnesium is necessary in order for the high affinity agonist-receptor complex to be induced (11, 40). In the absence of divalent cations, heterogeneity of agonist binding is not apparent and even agonist competition curves are steep, shifted to the right, and not affected by guanine nucleotides.

Of further interest are the binding patterns obtained with partial agonist drugs. It has been previously observed that the displacement curves of such agents in both the α_2 (platelet) and β-adrenergic systems are intermediate in their properties between those of antagonists and full agonists. Thus, the curves are intermediate in their slope factors and are shifted to the right by guanine nucleotides but less so than is observed for full agonists. In fact, in both systems it had previously been demonstrated that there is a high correlation between the "fold shift" in EC_{50} of an agonist displacement curve by guanine nucleotide, and the intrinsic activity of the drug for either activating or inhibiting adenylate cyclase (40, 44). The reason that such a correlation might exist is as follows. Computer modeling of competition curves by agonists and antagonists of varying intrinsic activities in the frog erythrocyte β-adrenergic receptor system was performed. An excellent correlation between the K_L/K_H ratio, or the percentage of receptors associated with the high affinity state ($\%R_H$) and the intrinsic activity of the drug was found ($p < 0.001$, $r = 0.95$). Put in other terms, to the extent that a drug is capable of inducing or stabilizing a high affinity nucleotide sensitive state of the receptor, to that extent will it be capable of interacting with the adenylate cyclase system as an agonist. Antagonists, which by definition have no intrinsic activity, do not appear to differentiate between different affinity states of the receptor (46).

A schema that accommodates these findings and relates them to adenylate cyclase activation (β receptors) or inhibition (α receptors) is as follows:

$$H + R \leftrightarrows HR + X \leftrightarrows HRX \overset{N}{\rightleftarrows} H + R + E_2(X).$$

In this scheme H = hormone or agonist, R = receptor, X = some effector component distinct from R, N = a guanine nucleotide such as GTP, and E_2 = an altered adenylate cyclase of either enhanced or depressed activity. We have previously referred to this model as a dynamic receptor affinity model for adenylate cyclase activation (11). This stresses the notion that the receptors are capable of existing in more than one discrete state which have strikingly different affinities for agonists. The initial step is the formation of a low affinity freely reversible complex of an agonist and receptor (HR).

This is followed by a second step in which the hormone receptor complex combines with some other component of the adenylate cyclase system denoted in the scheme as *X*. This ternary complex of hormone, receptor, and some other component is an intermediate on the pathway to adenylate cyclase activation. This intermediate is viewed as facilitating the activation of the enzyme by regulatory guanine nucleotide. For a variety of reasons, it seems plausible that the component of the adenylate cyclase system with which the *HR* complex combines is the nucleotide regulatory site (47). Guanine nucleotides, in a concerted process, are envisaged as dissociating the high affinity hormone receptor intermediate complex, reverting the receptor to lower affinity, and activating the adenylate cyclase. Antagonists presumably do not form this complex or at least do not perturb the equilibrium between existing states of the receptor since they appear to have homogeneous affinity for all receptors present. Partial agonists, whose K_L/K_H ratio is not quite so high as that of full agonists, induce the formation of less high affinity intermediate and hence produce less adenylate cyclase activation. The major point of this conceptualization is that agonists stabilize a high affinity intermediate complex which serves to facilitate activation of adenylate cyclase by regulatory guanine nucleotides. Interestingly, analogous mechanisms may exist for the α receptor wherein the high affinity complex serves to facilitate inhibition of the enzyme by nucleotides. The detailed mechanisms by which such opposite effects on the enzyme can be mediated by such analogous processes remain to be determined.

It has been observed that when such radioligand binding experiments are performed in whole cells, agonist competition curves are steep and shifted to the right even in the absence of added guanine nucleotides (46, 48, 49). Presumably this represents the effects of the high endogenous levels of intracellular guanine nucleotides. Thus, in vivo, with an intact cell, the high affinity intermediate which could be demonstrated in the membrane studies noted above is generally not observed. This means that the intermediate is, in fact, quite transient in its existence. A point to be further stressed is that the ability to demonstrate "shallow" agonist competition curves and effects of guanine nucleotides on these curves may depend on the extent to which membrane preparations are already contaminated with endogenous guanine nucleotides or perhaps other as yet unknown analogous factors. In those systems in which high concentrations of nucleotides are present in membrane fractions agonist curves may already appear to be shifted to the right and be unresponsive to added guanine nucleotides. This may lead to the erroneous conclusion that such mechanisms are not operative. In fact, the often quoted finding that turkey erythrocyte β-adrenergic receptors do not display a high affinity guanine nucleotide sensitive state may well be explained on this basis (9, 50).

Recently, Tolkovsky & Levitski et al have proposed a "collision coupling" theory for activation of adenylate cyclase by β-adrenergic receptors in the turkey erythrocyte membranes (50). This theory is based, in part, on the failure to observe a high affinity agonist-receptor complex even in isolated membranes. This theory proposes that activation of the enzyme by the β-receptor is due to the collision of the freely reversible complex H-R with the enzyme. Any intermediates formed are postulated to be so transient as not to be measurable. However, as noted above there is reason to believe that in the presence of guanine nucleotides the high affinity intermediate discussed above is in fact quite transient. Thus, the "collision coupling" theory (50) and the "dynamic receptor affinity" model (11) are really quite reconcilable. The high affinity intermediate postulated in the dynamic receptor affinity model and experimentally demonstrated in a number of systems is expected to be quite transient in vivo.

As noted above, very comparable findings have been observed for the α-adrenergic receptors. However, a complicating feature is that it appears that only the α_2 but not the α_1 receptors that have been so far studied are subject to agonist-specific regulation by guanine nucleotides (Hoffman, B. B.; Mullikin-Kilpatrick, D.; and Lefkowitz, R. J.; submitted for publication). This seems reasonable in view of the fact that thus far only the α_2 receptors appear to be closely associated with the enzyme adenylate cyclase (37, 38). Nucleotide regulation of the α receptors can be demonstrated by examining the ability of nucleotides to shift in an agonist-specific fashion the competition curves of drugs with an antagonist such as [^{3}H]DHE (40). Alternatively, the ability of guanine nucleotides to decrease [^{3}H]agonist, e. g. [^{3}H]epinephrine binding to the receptors, could be studied directly (51). However, since certain antagonists such as [^{3}H]WB4101 appear to label exclusively the α_1 receptors in some tissues, agonist competition with this blocker has not been found to be affected by guanine nucleotides (51).

Another interesting aspect of the molecular regulation of α-adrenergic receptors relates to the effects of monovalent cations such as sodium (52, 53). It has been demonstrated in certain model systems such as the α-adrenergic receptor of rabbit platelets (52) that sodium ion mediates an agonist-specific decrease in receptor binding affinity analogous to that originally reported for opiate receptors (54). Thus, displacement curves of agonists in competition with [^{3}H]DHE are shifted to the right by Na^+ and other monovalent cations in proportion to their intrinsic activities. Antagonist displacement curves are not affected. This effect also can be demonstrated directly by studying [^{3}H]epinephrine binding to α receptors in brain fractions (53). To date it is not clear whether this regulatory phenomenon is exclusively associated with only the α_2 receptors or whether both α_1 and α_2 receptors participate. It is also not clear what relation possible high and

low affinity states of the receptors might play in explaining these sodium-induced shifts in affinity. Whether mechanisms analogous to those described above for guanine nucleotide regulation are operative remains to be determined. Other regulators besides nucleotides and monovalent cations possibly exist which are capable of causing agonist-specific regulation and transitions between varying affinity states of α- and β-adrenergic receptors. The role that any such regulators might play in mediating coupling between the adrenergic receptors and their effector systems such as adenylate cyclase, ion channels, or others also remains to be elucidated.

REGULATION OF ADRENERGIC RECEPTOR NUMBER

Having considered some aspects of the molecular regulation of α and β receptors we now turn to the factors that regulate overall adrenergic receptor number. With the exception of desensitization very little is known about the cellular mechanisms that account for either increases or decreases in adrenergic receptor number in the variety of situations that have been examined. In Table 1 we list some of the many examples that have been studied. In the following we discuss a few of these areas.

Desensitization of Adrenergic Receptors

Of the various regulatory phenomena that involve the adrenergic receptors, perhaps the ones that have aroused the most investigative interest in recent years are those processes often referred to collectively as "desensitization." Under discussion here are a group of phenomena, undoubtedly involving a variety of mechanisms, which have several features in common. After a

Table 1 Some examples of regulation of α and β receptors[a]

Regulatory process	Receptor type	
	α	β
Desensitization	55–58	59–83
Hypersensitization	87	84–88
Hormones		
Thyroid	90, 97, 98, 103–105, 109, 111	89–103, 106–110
Steroids	34, 35, 113	35, 112–114
Drug-induced/Disease States	121, 125	115–120, 122–124, 126–131
Developmental/Aging		132–142

[a] As an overall guide to the already extensive literature on adrenergic receptor regulation, a listing of some of the studies examining selected areas of α or β receptor regulation is supplied.

cell or tissue has been exposed for a period of time to an agonist hormone or drug such as a catecholamine, that tissue often becomes less responsive to further stimulation by that particular agent. A variety of terms have been used to describe such processes including tolerance, desensitization, refractoriness, and tachyphylaxis. This phenomenon has clearly been demonstrated for at least some β-adrenergic and α-adrenergic responses. With the advent of direct radioligand binding studies interest has focused on the question of whether alterations in the adrenergic receptors themselves, induced by chronic exposure to agonist catecholamines, might in any way contribute to such desensitization phenomena. As is described below there is now rather strong evidence that receptor alterations do contribute to at least some forms of catecholamine-induced refractoriness. However, it is equally clear that certain forms of desensitization appear to involve alterations occurring mainly and perhaps exclusively distal to the receptors. Mixed mechanisms also appear to occur.

Perkins and colleagues have categorized such desensitization phenomena as being of two broad classes (70). They have referred to these as "heterologous" and "homologous" desensitization. Heterologous desensitization refers to a phenomenon induced in whole cells after chronic exposure to a biologically active hormone and is characterized by less responsiveness of the cell to a variety of other hormonal stimulators. Thus, consider a cell which normally responds to catecholamines through a β receptor mechanism and to prostaglandins through a separate prostaglandin receptor. Upon exposure to catecholamines such cells might become less responsive to both catecholamines and prostaglandins. This would be an example of heterologous desensitization. This type of desensitization has been demonstrated in several cell types. In some systems it has been demonstrated that analogues of cyclic AMP can themselves produce a comparable form of desensitization (70). In addition, Perkins and collaborators have shown that this form of desensitization does not appear to be associated with alterations in the β-adrenergic receptors as assessed by binding with the antagonist ligand iodohydroxybenzylpindolol (75). In some systems this form of desensitization appears to disappear when the cells are lysed and membrane fractions are prepared, but it is not yet clear whether this is a general characteristic of heterologous desensitization.

Taking these findings together it appears that heterologous desensitization represents a feedback mechanism in which catecholamine stimulation of β-adrenergic receptors leads to elevated levels of cyclic AMP, which are able to lead to an alteration distal to the hormone receptor binding sites which leads to a dampening of further cellular response. Inasmuch as cyclic AMP action is usually mediated through the stimulation of phosphorylation of proteins (143), it seems reasonable to speculate that the mechanism

of this form of desensitization is a cAMP-stimulated phosphorylation of some component of the adenylate cyclase system other than the receptor binding sites. This phosphorylation presumably leads to an inactivation of the component involved. If this speculation is correct, it is still not known what the potential locus of such a phosphorylation might be, although likely candidates at this point would be the nucleotide regulatory site(s) or the catalytic moiety itself.

A second broad class of desensitization phenomena that have been observed in β-adrenergic receptor systems is referred to as homologous desensitization (70). This refers to a situation where, after exposure to a β-adrenergic agonist for a period to time, the cell becomes refractory specifically to further β-adrenergic stimulation. Stimulation by other hormonal effectors such as prostaglandins would remain normal. An example of such a homologous form of desensitization can be demonstrated in the frog erythrocyte β-adrenergic receptor system. Frog erythrocytes are first exposed to the β-adrenergic agonist isoproterenol for several hours and then washed; then membrane fractions are prepared. The ability of isoproterenol to stimulate the enzyme adenylate cyclase through β-adrenergic receptors in membranes derived from the preincubated cells is found to be markedly reduced (63,64). The EC_{50}'s of the dose-response curves, however, are not significantly different. The ability of sodium fluoride and of the hormone prostaglandin E_1 to stimulate the enzyme from isoproterenol-desensitized cells is not significantly impaired. This is therefore an example of homologous desensitization. Conversely, if cells are preincubated with prostaglandin E_1 (144) the adenylate cyclase in membranes prepared from these cells becomes specifically desensitized to further prostaglandin stimulation, there being no effect on isoproterenol or fluoride stimulation. Very comparable forms of homologous desensitization occurring in β-adrenergic receptor systems have been demonstrated in a number of cell types including macrophages (61), cultured S49 lymphoma cells (69), lymphocytes (59), and several other cultured cell lines (62). These desensitization phenomena appear to be time-, concentration-, and temperature-dependent. Interestingly, only agonists but not antagonists promote this desensitization (63,72). Moreover, antagonists competitively antagonize the ability of agonists to desensitize the cells. Thus there is little doubt that the desensitization phenomena are in fact mediated through the β-adrenergic receptors themselves.

In contrast with the heterologous desensitization discussed above there is good evidence that receptor alterations are involved in the homologous form of desensitization. There appear to be at least two distinct types of changes involved. First, there is a striking fall in the amount of radiolabeled antagonist bound to the receptors in membranes derived from the desensitized cells. This is because, as has been shown elsewhere, there is a significant

fall in the number of assayable β-adrenergic receptors in these membranes (63, 64, 72). Second, there is at least one other alteration involving the receptor. This was initially suggested by findings with the radiolabeled β-adrenergic agonist ligand [^{3}H]HBI in which it was demonstrated that, on a percentage basis, the fall in [^{3}H]HBI binding after desensitization exactly paralleled the fall in catecholamine-stimulated adenylate cyclase (77, 83). In contrast, the fall in antagonist [^{3}H]DHA binding was somewhat less on a percentage basis than the fall in these other parameters. An explanation of these findings has been suggested from computer modeling of isoproterenol competition curves in membranes from desensitized cells (46). Not only is the number of β-adrenergic receptors diminished in the membranes from the desensitized cells but the ability to form the high affinity nucleotide-sensitive state of the receptor characteristic of agonist drugs is also diminished. This reduction in the formation of the high affinity state of the receptor is associated with a functional uncoupling of the receptors from productive adenylate cyclase activation. The fall in receptor number and the impairment of the ability to form a high affinity state of the receptor constitute two lesions in the receptors which appear to contribute to the diminished isoproterenol-stimulated adenylate cyclase which can be observed in the desensitized cell membranes (46).

The detailed molecular mechanisms responsible for these receptor alterations are not known at present. Several interesting speculations, however, may be considered. Since only agonists but not antagonists lead to desensitization it seems reasonable to speculate that the high affinity form of the receptor induced by agonists may be a crucial intermediate not only for activation of the enzyme but also for desensitization as well. The nature of the lesion responsible for the impairment of high affinity state formation is not known at present. At least in theory the lesion leading to impaired high affinity state formation could reside either in the receptor or in a more distal component such as the nucleotide regulatory site.

Another major question is the mechanism of the fall in β receptor number as assessed by radiolabeled antagonist binding. This could be due to a covalent or conformational alteration of the receptor which distorts the ligand binding site. However, in some systems it appears that the receptors may actually be lost from the membranes, perhaps by an internalization mechanism analogous to that recently described for polypeptide hormone receptors (145). At present it is an open question as to whether internalization of the β receptors in fact occurs and if it does whether it plays a role in lowering receptor number during the desensitization process. However, given the widespread occurrence of this internalization mechanism in other receptor systems it certainly would not be at all surprising if it were operative in catecholamine systems as well.

As noted above, homologous desensitization appears to be mediated through the β receptors themselves. That is, the receptors must be occupied in order for desensitization to occur. However, the receptor occupancy must be by an agonist or at least a partial agonist in order to observe desensitization. There is some evidence to suggest that more distal components of the system are also necessary for desensitization and that receptor occupancy by agonist is not sufficient. It has been observed that "down regulation" of β receptors by catecholamines does not occur in a variant of the S49 lymphoma cell which is termed AC– (69). This cell line is deficient in a nucleotide regulatory moiety of the adenylate cyclase (146). These data suggest that this component might be necessary as well. In the frog erythrocyte system desensitization appears to be blocked by reagents such as dicyclohexylcarbodiimide (147) which uncouple the receptors from the adenylate cyclase or by agents like NEM which destroy the adenylate cyclase catalytic activity (11, 148). Whether it is the actual physical presence of these distal moieties (nucleotide site and catalytic moiety) that is actually required or whether it is simply the generation of cyclic AMP in the presence of receptor occupancy by agonist which is necessary for desensitization is not yet clear. However, in at least one system, the AC– variant of the S49 lymphoma cell, agonist occupancy of the receptors even in the presence of added cyclic AMP still did not mimic the desensitization phenomenon (69).

An interesting set of observations concerning homologous desensitization has been reported by Su et al (82, 82a). Their data for the cultured astrocytoma line of cells appear to indicate a sequential mechanism in which adenylate cyclase desensitization occurs before a fall in β receptor number, assessed by IHYP binding. At this early stage it can be demonstrated that the ability of isoproterenol to displace IHYP from the β receptors is already markedly impaired, consistent with a lesion in formation of high affinity state receptors described above. Thus in this system the lesion involved in the formation of high affinity receptors appears to occur concomitantly with the desensitization of the enzyme but prior to the actual loss of receptors as assayed by IHYP binding. Su et al have suggested that the early lesion is a form of "uncoupling" which may lead to the subsequent loss of the receptors from the membranes. Whether such a sequential mechanism occurs in other cell types remains to be determined.

This discussion has focused on desensitization of β- rather than α-adrenergic receptors since a great deal more data are available in the former area. However, it should be pointed out that at least two examples of α-adrenergic receptor desensitization associated with alterations in ligand binding have been reported to date. One of these involves the α-adrenergic receptors coupled to a potassium efflux process in dispersed rat parotid

acinar cells (55). When these cells are incubated with epinephrine and then washed, they demonstrate a marked blunting in epinephrine-stimulated potassium efflux. This appears to be an example of homologous desensitization since the ability of muscarinic cholinergic agonists such as carbachol to stimulate potassium efflux remains unaltered. The desensitization is associated with a fall in [^{3}H]dihydroergocryptine binding to α-receptors in these cells. Both the desensitization and the fall in receptor binding can be blocked by including phentolamine, an α-adrenergic antagonist, in the initial preincubation with epinephrine.

In another example, when human platelets are incubated with epinephrine for a period of time the ability of α agonists to induce platelet aggregation is impaired whereas the ability of other agents to induce platelet aggregation remains unaffected (58). This refractoriness to epinephrine-induced aggregation is associated with a fall in the number of α-adrenergic receptors in the platelets as assessed by [^{3}H]DHE binding. To date, detailed studies of mechanisms have not been reported in these two α-adrenergic receptor systems.

Hyper- and Hypothyroidism

Some of the clinical signs of hyperthyroidism have suggested the possibility of altered responsiveness to catecholamines in this situation. The tachycardia, increased myocardial contractility, and skeletal muscle tremor often present in hyperthyroidism are reminiscent of marked β-adrenergic stimulation and can be reversed by β-adrenergic blockade. While not all the experimental work in this area is in agreement, there is suggestive evidence that there is an increased sensitivity to β-adrenergic agonists in a variety of tissues in the hyperthyroid state. For example, isolated mouse hearts in tissue culture exposed to T_3 become selectively more sensitive to the chronotrophic effect of β-adrenergic stimulation (91). One possible mechanism that could account for the increased sensitivity would be an increase in the number or affinity (or both) of β-adrenergic receptors. A substantial number of studies have examined this hypothesis with direct radioligand binding studies with varying results.

There is general agreement that there is an increase in the number of β receptors in membranes from hearts of hyperthyroid rats (94, 96, 98, 100).[3] Just how thyroid hormones cause this increase in β-adrenergic receptor number is unknown; the situation in hyperthyroid animals is particularly complex in view of possible effects of thyroid hormones on tissue

[3]McConnaughey, M. M., Jones, L. R., Watanabe, A. M., Besch, H. R. Jr., Williams, L. T., Lefkowitz, R. J. Thyroxine and propylthiouracil effect on alpha- and beta-adrenergic receptor number, ATPase activities, and sialic acid content of rat cardiac muscle vesicles. Submitted for publication.

catecholamines and other humoral factors which could influence β receptors indirectly. The increase in β receptor number in hyperthyroid hearts might be caused by a thyroid hormone-induced increase in receptor synthesis; this possibility is supported by the known direct augmentation of protein and phospholipid synthesis by T_3. Furthermore, β receptor number is increased by T_3 in cultured myocardial cells which suggests a direct effect of T_3 (95). Thyroid hormone also apparently increases the number of β-adrenergic receptors in isolated rat heart ventricle slices incubated in vitro (100). The increase in [^{3}H]dihydroalprenolol binding that developed over a 15-hr incubation in that study was inhibited by cycloheximide and was thought to be due to stimulation of the synthesis of new β-adrenergic receptors.

Changes in β receptor number have also been noted in other tissues in the hyperthyroid state. In the rat submaxillary gland there is an increase in β-adrenergic receptors (compared with the hypothyroid state) (Pointon, S. E. and Banerjee, S. P., submitted for publication). The situation in adipose tissue is unsettled. Malbon et al (99) reported no change in β-adrenergic receptor number in fat cell membranes derived from hyperthyroid rats with ~ 250 fmol/mg protein of β-adrenergic receptors in control and treated rats. This receptor density is in close agreement with the 240 fmol/mg protein found in euthyroid rat fat membranes by Williams et al (149). On the other hand Ciaraldi & Marinetti (103) reported nearly a doubling of β-adrenergic receptors in hyperthyroid rat fat membranes compared with controls (74 vs 40 fmol/mg respectively). In this latter study, however, detailed characteristics of the binding sites were not described and receptor number was apparently estimated with a single concentration of [^{3}H]DHA (15 mM) which would provide a less accurate estimate of receptor number than a full saturation curve.

The β-adrenergic receptor of the turkey erythrocyte has also been studied in hyperthyroidism. Bilezikian et al (108) noted no change in β-adrenergic receptor number or affinity, nor in isoproterenol-stimulated adenylate cyclase activity in broken cell preparations derived from erythrocytes of hyperthyroid turkeys. Interestingly, however, cAMP production in whole cells from hyperthyroid birds was more sensitive to catecholamines than in control cells.

Finally, in one clinical study (110) there was no change in β-adrenergic receptor number in lymphocytes from hyperthyroid patients compared with normal controls.

Conversely, in hypothyroidism, it has been suggested that there may be a diminished sensitivity to β-adrenergic stimulation. For example, in the hypothyroid rat, after the injection of isoproterenol there is reduced adrenergic responsiveness as assessed by changes in tail skin temperature (β receptor-mediated vasodilitation) and plasma glucose concentration (93).

There seems to be general agreement that in the hypothyroid heart there is a reduction in β-adrenergic receptor number. Ciaraldi & Marinetti reported a decrease in cardiac β-adrenergic receptors in hypothyroid rats compared with those from euthyroid animals (98, 103). However, in the earlier study the [^{3}H]DHA binding sites studied were not extensively characterized while in the latter study the 30% decrement in cardiac β-adrenergic receptors in hypothyroid rats seems to have been noted in a very limited number of experiments. Banerjee & Kung have also noted a reduction in β-adrenergic receptors in hearts from hypothyroid rats without change in receptor affinity for antagonists (96). Also, an apparent decrement in β-adrenergic receptor number was noted in cardiac membranes derived from hypothyroid sheep compared with normal controls (106). However, the [^{3}H]DHA binding sites were again not characterized in any detail and receptor concentration was estimated with a single concentration of [^{3}H]DHA (20 nM). The use of a single concentration of radioligand to estimate receptor number is to be discouraged since changes in receptor affinity and the increased nonspecific binding at high radioligand concentrations may both contribute to inaccurate estimates of receptor number. Most recently, McConnaughey et al[3] have confirmed the reduction in β-adrenergic receptor number (without change in affinity) in hypothyroid rat hearts.

A fall in β-adrenergic receptors has been noted in skeletal muscles from hypothyroid rats (102). Similarly, there is a reduction in β-adrenergic receptors in erythrocytes from hypothryoid turkeys (108). This is associated with decreases in both catecholamine-stimulated adenylate cyclase activity in broken cells and in cAMP accumulation in whole cells. Thyroidectomy leads to a reduction in β-adrenergic receptors in rat submaxillary glands (Pointon, S. E. and Banerjee, S. P., submitted for publication). There is agreement from two laboratories that there is no change in β-adrenergic receptor number in fat cells from hypothyroid rats (99, 103). However, a decrease in β-adrenergic receptor number in fat from thyroidectomized rats has recently been reported (107).

There has also been a great deal of interest in assessing by radioligand binding possible changes in α receptors in altered thyroid states. This has been motivated by the results of pharmacological experiments that demonstrate increased α-adrenergic sensitivity in the hearts of hypothyroid animals (90, 105). While several studies have appeared using [^{3}H]dihydroergocryptine to measure α receptor number and affinity in altered thyroid states, there is not agreement as to what changes, if any, are present in mammalian hearts. The α receptor populations of hearts from hypothyroid rats have been directly studied by four groups. The initial report of Ciaraldi & Marinetti (98) noted a reduction in α receptors in the hearts of propylthiouracil-treated rats compared with euthyroid controls. However, the binding sites were not characterized in detail and were present

in numbers manyfold greater than found by other groups. In a later study (103) this group again reported a decrement in α receptor number in hypothyroid hearts with the receptors apparently having increased affinity for [^{3}H]DHE. Sharma & Banerjee (104) reported an increase in α receptor number in thyroidectomized rats compared with T_3-treated animals. A precise conclusion as to the effect of hypothyroidism is difficult in view of the absence of a euthyroid control group. Williams & Lefkowitz (111) report no change in either α receptor number or affinity in hearts from propylthiouracil-treated or thyroidectomized rats compared with normal rats. Using a different membrane preparation, McConnaughey et al[3] found a decrement in α receptor number with no change in affinity.

There is also disagreement about possible changes in α receptors in hyperthyroid rat hearts. Ciaraldi & Marinetti (98, 103) noted a decrement in both receptor number and affinity compared with euthyroid controls. Williams & Lefkowitz reported a decrease in [^{3}H]DHE binding affinity for α receptors with an insignificant reduction in receptor number (111). McConnaughey et al[3] noted a reduction in receptor number with no change in binding affinity. Thus definite conclusions about α receptor changes in hearts from hypo- and hyperthyroid rats are not possible based on the available data. Some of the reasons for the discrepant findings might include the different treatment schedules and membrane preparations used by the various groups. The details of the membrane preparation and consequent receptor recovery are particularly important in view of the demonstration that the α receptor population in rat heart is probably a mixture of α_1 and α_2 receptor subtypes (150). Indeed, an important refinement in analysis would be to reassess possible α receptor regulation in rat heart by thyroid hormone by quantifying α receptor subtypes in treated animals.

Another area of potentially fruitful investigation would be to study the liver with radioligand binding techniques to measure α and β receptors in altered thyroid states. It has recently been demonstrated, using isolated rat hepatocytes, that in euthyroid animals phosphorylase a activation by catecholamines has a potency series characteristic of an α receptor–mediated effect whereas in the hypothyroid state the activation seems to be mediated via β receptors (109). This is opposite to the dominance of α-mediated effects seen in heart and fat in hypothyroidism. Radioligand binding studies would add an important dimension to our understanding of this system.

In summary, there are many examples of an increase in β-adrenergic receptors and a fall in α receptors in hyperthyroidism, and of a fall in β-adrenergic receptors and a rise in α receptors in hypothyroidism. However, generalizations are not possible at this point because of conflicting data and exceptional examples. In any case, these data do not really test the challenging hypothesis that α and β receptors are interconvertible entities (151, 152).

Adrenalectomy

Adrenalectomy has complex effects on the pharmacological response of rat liver to catecholamines. The possible role of changes in adrenergic receptors themselves in this phenomenon has been evaluated in two studies. Wolfe et al (112) found a three to five fold increase in β receptor number in adrenelectomized rat liver membranes compared with controls; this change was reversed by treating the adrenelectomized rats with cortisone. Guellaen et al (113) found a very similar increase in β receptor number in adrenalectomized rat liver membranes; this group also measured α receptors in these membranes and found them to be not different from controls. This suggests that α and β receptors in rat liver plasma membranes may be under independent regulation with respect to glucocorticoids. The role of glucocorticoids in regulating adrenergic receptor number in other tissues remains to be explored and such studies in disease states where steroids have therapeutic benefit (i.e. asthma) would be of interest.

Disease-Associated Changes in Adrenergic Receptors

Whereas much work has been done relating changes in, for example, cholinergic and insulin receptors to disease states, relatively little information is available on changes in α- and β-adrenergic receptors in disease states characterized by possibly abnormal sympathetic nervous system function. The available information relates predominantly to abnormalities found in circulating blood cells. Cells such as polymorphonuclear leukocytes and lymphocytes have been shown by direct binding studies to contain typical β-adrenergic receptor binding sites (153). These appear to be coupled to adenylate cyclase. Such cells provide a convenient source for clinical studies of β-adrenergic receptor function. Similarly, α-adrenergic receptors have been demonstrated by binding techniques on human platelets (37). These appear to inhibit adenylate cyclase activity and mediate the effects of α agonists on platelet aggregation. The greatest effort has been directed toward understanding β-adrenergic mechanisms in asthma, stimulated by the hypothesis that a defect of the β receptor may play a major role in the etiology of this disease (154). It is clear that human leukocytes have a diminished cAMP production (desensitization) both in vivo and in vitro when exposed to β agonists for prolonged periods (115, 116, 120). While it is likely that desensitization of leukocytes is associated with a reduction in β receptor number (129), it is uncertain whether or not untreated asthmatics have abnormalities in their leukocyte β receptors (129, 130). Even if they do, the relevance of any such abnormalities to receptor alterations in their airways would still need to be established.

The putative "propranolol withdrawal syndrome" in man relates to the possible supersensitivity of patients to β agonists when propranolol is

abruptly discontinued; this may be manifest by severe worsening of angina in patients with coronary artery disease. While increased β receptor numbers have been documented in the hearts of rats treated chronically with propranolol (85) and denervated with guanethidine (88), the relation, if any, of these findings to the clinical syndrome is unknown. Recently, Nies et al (131) reported an increase in β receptor number in leukocytes from patients receiving propranolol compared with untreated controls; in that study the actual number of β receptor sites was not reported.

SUMMARY

Since the relatively recent identification of α- and β-adrenergic receptors by radioligand binding, significant strides have been made in understanding the molecular and physiological regulation of these receptors. The importance of ions and nucleotides in regulating both α and β receptor affinity is now appreciated and some of the details of their effects have been explored. The quantification of receptor subtypes by ligand binding opens new opportunities for research in the regulation of receptor number. While hormones and other factors have been clearly demonstrated to regulate receptor number, little is known about the cellular mechanisms controlling adrenergic receptor turnover which presumably mediate these changes. This will likely be a fruitful area for future research.

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