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IN VITRO INTERACTIONS OF AGONISTS AND ANTAGONISTS WITH β-ADRENERGIC RECEPTORS*

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Abstract—Neurotransmitters and hormones mediate their effects through interaction with specific receptors. A complete understanding of the effects of these chemical signals requires detailed knowledge, at the molecular level, of agonist/receptor interactions. It is likely that agonists and antagonists interact with the same site on a receptor. Agonists, however, are by definition different from antagonists in that agonists are responsible for transducing information across the cell membrane, ultimately resulting in a biological response, while antagonists appear to act through passive occupancy of receptors. Implicit in this concept is the idea that these fundamental differences between agonists and antagonists arise from the sequelae induced by agonist-specific interactions with receptors.

The development of *in vitro* radioligand binding assays that permit direct study of β-adrenergic receptors has led to a wealth of information on the distribution, density, and regulation of these receptors [1, 2]. Such assays have also allowed more direct examination of initial agonist/receptor interactions than was possible with assays employing intact physiological systems or biochemical effector systems. Unfortunately, it is often difficult to distinguish an agonist from an antagonist using the *in vitro* binding of radiolabelled ligands. In some situations, however, such assays are capable of identifying differences between the interactions of receptors with agonists as opposed to antagonists. These differences are presumably due to the fundamentally different ways in which agonists and antagonists interact with the receptor. This report presents a brief summary of some of the *in vitro* differences between agonist/receptor and antagonist/receptor interactions (Table 1). A schematic model of the β-adrenergic receptor-coupled adenylate cyclase system, including many of the *in vitro* consequences of agonist/receptor interactions, is shown in Fig. 1.

EFFECTS OF GTP AND Mg^{2+} ON AGONIST AND ANTAGONIST INHIBITION OF THE BINDING OF RADIOLABELLED ANTAGONISTS

β-Adrenergic receptors are usually studied by employing one of a number of radiolabelled antagonists such as (^{3}H)-DHA‡ [3], (^{125}I)-IHYP [4, 5], or (^{125}I)-IPIN [6]. This allows direct quantitation of the affinity of the receptor for each radiolabelled antagonist and of the density of receptors. The affinity of the receptor for unlabelled ligands can be determined indirectly by assessing the ability of these ligands to inhibit the binding of a radiolabelled antagonist.

Magnesium and GTP are important cofactors required for stimulation of adenylate cyclase activity by a variety of different agonists including catecholamines, peptides, and prostaglandins [7]. These cofactors also affect the apparent affinity of β-adrenergic receptors for agonists. In the absence of guanine nucleotides, inhibition of the binding of radiolabelled antagonists by agonists is complex, characterized by shallow inhibition curves with Hill coefficients typically in the range of 0.5–0.7 (Fig. 2). In the presence of GTP, GDP, or stable analogues of GTP such as Gpp(NH)p or GTPγS, these inhibition curves are shifted to the right and Hill coefficients approach a value of 1.0. Antagonists, on the other hand, display simple competitive interactions with the receptor and inhibition curves are generally unaffected by guanine nucleotides (Fig. 2). The effects of guanine nucleotides on the affinity of β-adrenergic

Table 1. Summary of agonist-specific phenomena at β-adrenergic receptors

(1)	Agonists increase adenylate cyclase activity.
(2)	Guanine nucleotides decrease the affinity of the receptor for agonists.
(3)	Divalent cations increase the affinity of the receptor for agonists.
(4)	The interactions of agonists are driven by a decrease in enthalpy ($\Delta H° < 0$) while the interactions of antagonists are entropy-driven ($\Delta S° > 0$).
(5)	Agonists induce a conformational change in the receptor which exposes a reactive sulfhydryl group.
(6)	Agonists cause a decrease in the affinity of the receptor on intact cells for agonists.
(7)	In intact cells agonists cause the receptor to uncouple from the adenylate cyclase system and ultimately cause a decrease in the density of membrane-bound receptors.

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‡ Abbreviations: (^{3}H)-DHA, (^{3}H)-dihydroalprenolol; (^{125}I)-IHYP, (^{125}I)-iodohydroxybenzylpindolol; (^{125}I)-IPIN, (^{125}I)-iodopindolol; Gpp(NH)p, guanosine-5′-(β-imido)triphosphate; GTPγS, guanosine-5′-(3-*O*-thio)triphosphate; G/F, the regulatory component of adenylate cyclase; WT and CYC$^-$, the wild type and adenylate cyclase-deficient variant of the S49 lymphoma cell; (^{3}H)-HBI, (^{3}H)-hydroxybenzylisoproterenol; (^{3}H)-EPI, (^{3}H)-epinephrine; NEM, *N*-ethylmaleimide.

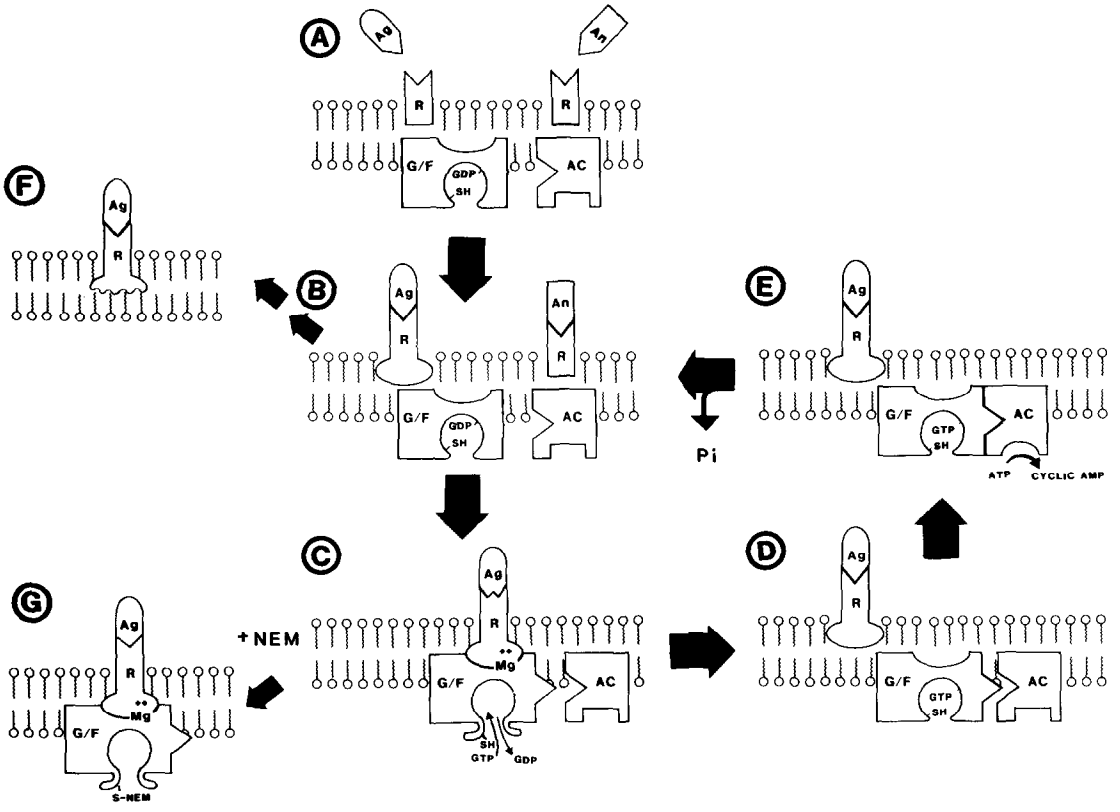

Fig. 1. Schematic model of the β-adrenergic receptor-coupled adenylate cyclase system. Antagonists (An) are thought to occupy the receptor (R) site without inducing a conformational change in the receptor (A). Agonists (Ag), however, interact with the receptor, inducing a conformational change (B) such that it is able to interact with the G/F protein (G/F) to form a high-affinity ternary complex composed of agonist, receptor, and G/F protein (C). This interaction induces a conformational change in the G/F protein that allows exchange of GTP for GDP, and exposes a reactive sulfhydryl group (C). In membranes, the newly exposed sulfhydryl group is now accessible to the alkylating reagent NEM (G). The insertion of GTP into the G/F protein destabilizes the ternary complex (D). The GTP-activated G/F protein can now interact with the catalytic unit of adenylate cyclase to form an active catalytic complex that converts ATP into cyclic AMP (E). Catalytic activity is terminated by the hydrolysis of GTP to GDP, and the system returns to the basal state. In intact cells exposure to agonists causes desensitization (F).

receptors for agonists have been observed in a variety of mammalian tissues [8]. The effects are similar in tissues that contain predominantly β_1 receptors, such as the heart, and in tissues that contain predominantly β_2 receptors, such as the lung. Thus, like the stimulation of adenylate cyclase activity, the effect of GTP appears to be conserved in both receptor subtypes.

There are many possible explanations for low Hill coefficients [9], including negative cooperativity, coexistence of independent receptor subtypes, and a two-step/three-component reaction [10, 11]. In the latter case, the agonist (H) first binds to the receptor (R) to form a relatively low-affinity hormone/receptor complex (HR) (Fig. 1B).

$$H + R \overset{K_1}{\rightleftharpoons} HR$$

This complex then interacts with a second membrane component (X) to form a high-affinity ternary complex (HRX) (Fig. 1C).

$$HR + X \overset{K_2}{\rightleftharpoons} HRX$$

This two-step model requires the existence of only one species of receptor. The receptor, however, can exist in two different states with distinct affinities for agonists. The extent of formation of the high-affinity ternary complex is limited by the affinity of HR for X and by the concentration of X in the membrane relative to that of R. The affinity of HR for X and the relative stoichiometry of R and X can affect the apparent affinity of the receptor for the agonist and the shape of the agonist displacement curve. In general, the Hill coefficient will be lower if the affinity of HR for X is high and if the concentration of X is less than that of R. For the β-adrenergic receptor, this two-step model has been compared with other potential kinetic models [12]. It appears to be the most appropriate explanation of the low Hill coefficients observed in studies of the ability of agonists to inhibit the binding of a labelled antagonist. The agonist-specific effects of guanine nucleotides are also best explained by this model.

Guanine nucleotides are thought to destabilize the high-affinity ternary complex by reducing the affinity of HR for X (Fig. 1D), such that, in the presence of

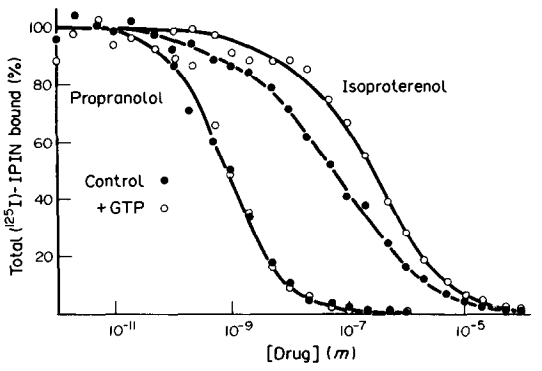

Fig. 2. Inhibition of the binding of (^{125}I)-IPIN to membranes prepared from WT S49 lymphoma cells by (−)-propranolol or (−)-isoproterenol in the presence or absence of GTP. Membranes prepared from S49 lymphoma cells (6 μg/tube) were incubated with (^{125}I)-IPIN (60 pM) and either (−)-propranolol or (−)-isoproterenol in the presence or absence of 100 μM GTP. The assay was carried out in a volume of 250 μl containing 20 mM HEPES and 1 mM $MgCl_2$. The reaction was incubated for 120 min at 22° and was terminated by the addition of 10 ml of wash buffer (10 mM TRIS, 1 mM $MgCl_2$). Membranes were collected by vacuum filtration through Schleicher and Schuell No. 30 glass fiber filters. Filters were washed with an additional 10 ml of wash buffer.

GTP, the ternary complex does not accumulate. Therefore, in the presence of GTP, the binding of agonists more closely conforms to a simple bimolecular reaction with a Hill coefficient of 1.0, and the apparent equilibrium affinity constant is that of the initial binding reaction.

The two-step model described above assumes that the β-adrenergic receptor can interact with a second membrane component, but the model makes no assumptions as to the identity of the proposed second membrane component. The effect of guanine nucleotides on the apparent affinity of the receptor for agonists, however, suggests that this component might be the G/F protein that is involved in agonist stimulation of adenylate cyclase activity. A variety of genetic and biochemical evidence is consistent with this suggestion. Genetic evidence has come from Gilman and coworkers [13], who have shown that effects of GTP on the affinity of the receptor for agonists do not occur in mutants of S49 lymphoma cells (CYC^-) which are missing a functional G/F protein. Limbird *et al.* [14] have reported that in the presence of agonists, but not antagonists, receptors interact with a (^{32}P)-ADP-ribosylated protein such that the apparent molecular weight of the hormone/receptor complex is increased. It also appears that in the presence of agonists, but not antagonists, the receptor forms a stable complex with a protein that is capable of conveying nucleotide-dependent adenylate cyclase activity to a preparation of the catalytic component of adenylate cyclase [15]. Thus, there is good kinetic, genetic, and biochemical evidence to support the hypothesis that agonists, but not antagonists, interact with the receptor to form a high-affinity ternary complex composed of agonist, receptor, and the G/F protein of adenylate cyclase (Fig. 1C).

Indirect radioligand binding studies have also shown agonist-specific effects of divalent cations on the affinity of the receptor for agonists [16]. In contrast to GTP, Mg^{2+} appeared to increase the apparent affinity of the receptor for agonists. This effect of Mg^{2+} was agonist-specific since there was little effect of Mg^{2+} on the affinity of the receptor for antagonists. The absence of this effect in membranes prepared from CYC^- cells suggests the need for a functional G/F protein. It is not known at the present time whether the site of action of Mg^{2+} is on the receptor itself or on the G/F protein (Fig. 1C).

The effects of GTP and Mg^{2+} are agonist-specific and can be detected with *in vitro* radioligand binding assays. Interestingly, there have been isolated reports that both GTP and Mg^{2+} can affect the apparent affinity of the β-adrenergic receptor for antagonists. In membranes prepared from L6 myoblasts, GTP increased the affinity of the receptor for antagonists [17]. In membranes prepared from rat lung and S49 lymphoma cells, Mg^{2+} decreased the affinity of the receptor for antagonists [18]. It is important to note that in these cases the effects of GTP and Mg^{2+} on the interactions of antagonists with the β-adrenergic receptor were in the opposite direction from their effects on the interactions of agonists with the receptor.

THERMODYNAMIC DIFFERENCES BETWEEN AGONISTS AND ANTAGONISTS

Pike and Lefkowitz [19] and U'Prichard *et al.* [20] reported that the ability of agonists to inhibit the binding of radiolabelled antagonists to the β-adrenergic receptor was enhanced by decreasing the temperature of the assay. In contrast, the affinity of the receptor for antagonists was relatively unaffected by changes in temperature. Detailed thermodynamic studies have allowed calculation of the changes in entropy ($\Delta S°$) and enthalpy ($\Delta H°$) associated with the interactions of agonists and antagonists with the receptor. In turkey erythrocytes [21], the binding of antagonists was temperature independent and was shown to be almost completely driven by increases in entropy. In marked contrast, the binding of agonists was temperature dependent and associated with decreases in enthalpy and thermodynamically unfavorable decreases in entropy. Therefore, the binding of agonists was driven by favorable decreases in enthalpy. These thermodynamic properties of agonists appeared to be important since the magnitude of the changes in both enthalpy and entropy were correlated with the efficacies of a number of full and partial agonists.

The above thermodynamic study [21] used turkey erythrocytes as a model system. Although this system has been a useful model, turkey erythrocyte β-adrenergic receptors have pharmacological and kinetic properties which differ from those of mammalian β-adrenergic receptors [22]. These observations make it difficult to generalize results obtained with turkey erythrocytes. Studies with four different mammalian tissues, however, revealed that the affinity of receptors for agonists increased as the temperature was decreased [23]. This was an agonist-specific effect since the affinity of receptors for antagonists re-

mained relatively unaffected by temperature. In tissues where GTP affected the affinity of receptors for agonists (rat heart, lung, and cerebellum), agonists were less affected by temperature in the presence of GTP than in its absence.

Calculation of thermodynamic parameters associated with the binding of ligands to β-adrenergic receptors in mammalian tissues revealed that decreases in enthalpy provided most of the driving force for the binding of agonists. Interactions of antagonists, however, were driven by increases in entropy. The finding that the energetics of the interactions of agonists with both β_1- and β_2-adrenergic receptors were similar indicates that these fundamental properties of β-adrenergic receptors are conserved in both receptor subtypes. In the heart, lung, and cerebellum, the binding of agonists was associated with less negative changes in enthalpy and entropy in the presence of GTP than in its absence.

The energetics of the binding of antagonists to β-adrenergic receptors are thermodynamically similar to those of many other protein-binding reactions in which information transfer is not involved [24]. These reactions are thought to be driven by hydrophobic interactions, the driving force being the increases in entropy which result when water molecules, ordered around the ligand and the ligand binding site, are displaced. Since binding reactions in general are associated with large positive changes in entropy, it is reasonable to suggest that the initial interaction of agonists with the receptor also results in an increase in entropy. The observed net decrease in entropy associated with the binding of agonists may reflect a conformational change either in the receptor or in surrounding membrane components (Fig. 1B). The fact that the efficacy of different agonists was correlated with their observed decreases in entropy suggests that the more efficacious an agonist is at inducing a conformational change in the receptor, the better it is at transferring information across the cell membrane. The observation that the addition of GTP was associated with less negative changes in entropy suggests that the formation of a ternary complex is responsible for at least some of the proposed conformational changes in the receptor (Fig. 1C).

DIRECT STUDIES OF AGONIST/RECEPTOR INTERACTIONS

Differences in the energetics of the interactions of agonists and antagonists with the β-adrenergic receptor, as well as differences in the effects of guanine nucleotides and divalent cations, suggest that there are fundamental differences between the interactions of agonists and antagonists with the receptor. These differences can be detected indirectly *in vitro* using radioligand binding assays with radiolabelled antagonists. Direct assays of β-adrenergic receptors with radiolabelled agonists offer an independent method of studying the properties of agonist/receptor interactions. Direct assays are potentially more sensitive than indirect competition studies in which the ability of agonists to inhibit the binding of a labelled antagonist is assessed.

Successful attempts to study β-adrenergic receptors with radiolabelled agonists have employed either (^{3}H)-HBI or (^{3}H)-EPI. These ligands are full agonists and are relatively potent compared with other agonists. (^{3}H)-HBI has been used in a number of different systems including tissues with either β_1-[25] or β_2-receptors [26–28]. In membranes prepared from rat lung, the density of receptors determined with either the agonist (^{3}H)-HBI or the antagonist (^{125}I)-IHYP was the same, indicating that (^{3}H)-HBI is capable of identifying the total population of receptors [27]. However, Scatchard analysis of the binding (^{3}H)-HBI revealed a marked curvature. This was in agreement with predictions based on the low Hill coefficients of agonist-inhibition of radiolabelled antagonists in this tissue. These results are also consistent with the hypothesis that both the high-affinity ternary complex and the low-affinity initial hormone/receptor complex were detected with (^{3}H)-HBI. The binding of (^{3}H)-HBI to the receptor was markedly inhibited by Gpp(NH)p. The effect appeared to be due mainly to a decrease in the amount of the high-affinity component. This result is consistent with the suggestion that guanine nucleotides decrease the affinity of HR for G/F, thereby preventing accumulation of the high-affinity ternary complex.

Studies of the kinetics of agonist/receptor interactions are difficult to carry out using indirect competition assays. The availability of (^{3}H)-agonists has facilitated determination of the kinetic parameters of agonist/receptor interactions [27]. In membranes prepared from rat lung, the dissociation kinetics of (^{3}H)-HBI were complex and there were marked effects of guanine nucleotides. In the absence of guanine nucleotides most of the specifically bound (^{3}H)-HBI dissociated at a very slow rate ($t_{1/2} \approx 3$–5 h). A small proportion, however, dissociated very rapidly ($t_{1/2} < 1$ min). In the presence of Gpp(NH)p the majority of the specifically bound (^{3}H)-HBI dissociated within about 1 min. The complex dissociation pattern in the absence of Gpp(NH)p is consistent with the nonlinear Scatchard plots observed in this tissue. The inhibition of the binding of (^{3}H)-HBI by Gpp(NH)p is apparently due to an extremely rapid and effective increase in the dissociation rate of the agonist.

(^{3}H)-EPI has also been used in studies of β-adrenergic receptors. U'Prichard *et al.* [20] used this ligand to study receptors in calf cerebellum and rat lung. Although the percentage of specifically bound (^{3}H)-EPI was relatively low, the binding had all of the properties expected of a high-affinity interaction with a G/F protein-associated form of the β-adrenergic receptor. That is, the binding of (^{3}H)-EPI was stereoselectively inhibited by both agonists and antagonists, had the appropriate pharmacological profile, and was inhibited by guanine nucleotides. Scatchard analysis of results obtained with (^{3}H)-EPI revealed apparently linear plots. This result suggests that (^{3}H)-EPI identifies only the high-affinity ternary complex. The low-affinity initial hormone/receptor complex apparently dissociates too rapidly to be trapped by filtration and is not identified by (^{3}H)-EPI.

Recently we have utilized a high specific activity, optically pure preparation of (^{3}H)-EPI to study β-adrenergic receptors on membranes prepared from L6 myoblasts (Fig. 3) [29]. Scatchard plots of the binding of (^{3}H)-EPI to L6 membranes were linear

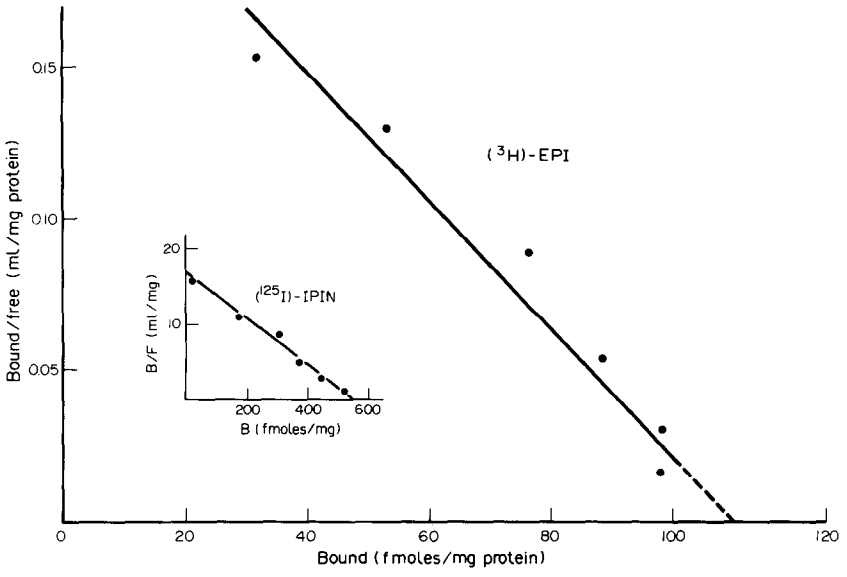

Fig. 3. Scatchard plots for the binding of (^{3}H)-EPI and (^{125}I)-IPIN to membranes prepared from L6 myoblast cells. Membranes prepared from L6 cells (198 μg/tube) were incubated with (^{3}H)-EPI (0.1–3.2 nM), 20 mM HEPES, and 1 mM $MgCl_2$ in a final volume of 1 ml for 120 min at 22°C. Specific binding was defined as binding inhibited by 1 μM propranolol and represented 82–45% of total binding. The K_{app} was 0.239 nM and the B_{max} was 111 fmoles/mg protein. Inset: The same membranes (4.4 μg/tube) were incubated with (^{125}I)-IPIN (1.0–200 mM), 20 mM HEPES, and 1 mM $MgCl_2$, in a final volume of 250 μl for 120 min at 22°. Specific binding was defined as binding inhibited by 50 μM isoproterenol. The K_d was 35.2 nM and the B_{max} was 539 fmoles/mg protein.

even when carried out with concentrations of (^{3}H)-EPI 20 times greater than the apparent K_d of the receptor for (^{3}H)-EPI. This result is consistent with the hypothesis that (^{3}H)-EPI identifies only the high-affinity ternary complex, and suggests that (^{3}H)-EPI is an appropriate radioligand to selectively study the β-adrenergic receptor as part of the ternary complex. As shown in Fig. 3, the apparent B_{max} for (^{3}H)-EPI was only about 20% of that of the antagonist (^{125}I)-IPIN. Thus not all of the receptors were able to participate in the formation of this high-affinity form of the receptor. The possibility that this is due to an unfavorable K_2 (see reaction scheme above) or to stoichiometrically limiting G/F is currently under investigation.

The effects of Mg^{2+} on the binding of (^{3}H)-agonists to the β-adrenergic receptor are readily apparent. Magnesium has been shown to increase the amount of (^{3}H)-HBI bound to the receptor, while it had no effect on the binding of the antagonists (^{3}H)-DHA [30] or (^{125}I)-IHYP [31]. This effect of Mg^{2+} has been shown to be due to an increase in the number of high-affinity sites for (^{3}H)-HBI, with no apparent change in the affinity of these sites for (^{3}H)-HBI [31].

EFFECTS OF SULFHYDRYL REAGENTS

Reagents that specifically modify different reactive groups on proteins are often used to determine the importance of these groups for protein or enzyme function. For instance, the sulfhydryl alkylating reagent NEM has been used to demonstrate the importance of sulfhydryl groups for β-adrenergic receptor-mediated stimulation of adenylate cyclase activity [32]. Bottari *et al.* [33] first demonstrated *in vitro* an agonist-specific effect of NEM on β-adrenergic receptors. These investigators demonstrated that pretreatment of receptors with NEM alone, an agonist alone, or NEM plus an antagonist had no effect on either the affinity of the receptors for (^{3}H)-DHA or the density of the receptors. However, preincubation of receptors with NEM plus an agonist resulted in a decrease of 50% in the density of receptors with no change in the affinity of the remaining receptors for (^{3}H)-DHA. Involvement of the G/F protein was suggested by the finding that GTP prevented or reduced the apparent decrease in the density of receptors [34]. The observation that the effect occurred in WT S49 lymphoma cells but not in CYC^- cells offered further evidence for the involvement of the G/F protein [35].

The apparent decrease in the density of β-adrenergic receptors after preincubation with NEM and an agonist was thought to involve an agonist-specific conformational change in the receptor which exposed an otherwise unavailable sulfhydryl group to irreversible alkylation by NEM [33]. The site of this alkylation was thought to be in or near the ligand binding site, thereby destroying the ability of receptors to bind (^{3}H)-DHA. Recent experiments with (^{3}H)-agonists have led to a somewhat different interpretation of the effect of NEM [31, 36]. Preincubation of membranes with NEM and (^{3}H)-HBI resulted in a significant fraction of the specifically bound (^{3}H)-HBI becoming persistently bound to the receptor [31]. Pretreatment also resulted in a loss of the ability of GTP to initiate dissociation of the agonist from

the receptor. Thus pretreatment of the receptor with NEM and an agonist did not inactivate the ligand binding site of the receptor, but rather caused the agonist to remain persistently bound in the ligand binding site (Fig. 1G).

β-ADRENERGIC RECEPTORS ON INTACT CELLS

All of the studies described thus far were performed with membranes prepared by homogenization of various tissues. These preparations offer a simplified, cell-free system in which the concentrations of cofactors such as GTP and Mg^{2+} can be easily manipulated. A great deal has been learned about the interactions of agonists and antagonists with β-adrenergic receptors through examination of these simplified systems. Nonetheless, β-adrenergic agonists and antagonists interact *in vivo* with living cells, not membrane fragments. β-Adrenergic receptors on intact cells possess properties not found in simple membrane preparations. For example, exposure of tissues or cells to agonists, but not antagonists, can initiate the process of desensitization [37] (Fig. 1F). In some systems this has been characterized as a two-step process, with an initial decrease in the responsiveness of adenylate cyclase, followed by a reduction in the number of receptors [38].

The results of studies of the binding of agonists and antagonists to β-adrenergic receptors on intact cells have been reported in several systems [39–41]. A common finding was that the interactions of receptors with agonists were very different in living cells as compared with membranes derived from the same cells. The affinities of receptors for agonists were generally 2–3 orders of magnitude lower when binding studies with radioligands were carried out with intact cells as opposed to membranes. The affinities of receptors for antagonists, however, were approximately the same in cells and membranes. Thus, not only were the properties of receptors on intact cells shown to be very different from those on membranes, but the differences were shown to be agonist-specific.

β-Adrenergic receptors on intact cells appear to have a low affinity for agonists at equilibrium. Pittman and Molinoff [41] investigated the time course of the binding of (^{125}I)-IHYP to intact L6 myoblasts and concluded that the properties of the receptor were changing during the course of the assay. At short times (<10 min), the binding of (^{125}I)-IHYP to intact L6 myoblasts was inhibited by 5 μM isoproterenol. At longer times, this concentration of isoproterenol was no longer able to inhibit the binding of (^{125}I)-IHYP. Thus it appeared that the affinity of receptors on intact L6 myoblasts for agonists was initially high but decreased within the first few minutes of incubation. This effect occurred only in intact cells and was agonist-specific.

More recently this phenomenon has been investigated in detail in intact S49 lymphoma cells [42, 43]. At equilibrium, the affinity of receptors for antagonists was similar in intact cells and membranes. Agonists, however, appeared much less potent in intact cells than in membranes. The kinetics of this phenomenon were investigated by performing competition experiments under nonequilibrium conditions. It was found that at very short times agonists were relatively potent but there was a rapid ($t_{1/2}$≈2–3 min) and marked (10- to 100-fold) decrease in the affinity of the receptor for agonists. It was proposed that this phenomenon was due to an agonist-promoted conformational change in the receptor. Since this phenomenon occurred in both WT and CYC$^-$ cells, a requirement for cyclic AMP or a functional G/F protein is unlikely.

A related series of experiments revealed that agonists also cause a time-dependent sequestration of receptors away from the cell surface [43]. WT cells were preincubated with agonists for short times (< 1 min) and then the ability of different ligands to inhibit the binding of (^{125}I)-IPIN in 1-min nonequilibrium competition experiments was determined. In the control case, with no preincubation, hydrophilic ligands, including the agonist isoproterenol and the antagonist sotalol, were only able to inhibit 80% of the specifically bound (^{125}I)-IPIN. In contrast, lipophilic ligands, including the agonist salbutamol and the antagonist propranolol, were able to inhibit all of the specifically bound radioligand. The observation that the ability of ligands to completely inhibit bound (^{125}I)-IPIN was related to their hydrophobicity suggested that the plasma membrane restricted the access of hydrophilic ligands from a pool of intracellular or sequestered receptors. The percentage of total receptors in this sequestered pool was increased by preincubation of intact cells with agonists. Preincubation of intact CYC$^-$ cells with agonists also led to an increase in the percentage of sequestered receptors. Therefore, it seems unlikely that either cyclic AMP or a functional G/F protein are required in the process of sequestration. Similar conclusions have been reached by Staehelin *et al.* [44] who used a radiolabelled hydrophobic antagonist ((^{3}H)-CGP 12117) thought to bind only to β-adrenergic receptors on the surface of cells.

CONCLUSIONS

The interactions of an agonist with its receptor are by definition different from the interactions of an antagonist with the same receptor. *In vitro* radioligand binding assays have uncovered a number of characteristics of agonist/receptor interactions that are not shared by antagonists (Table 1). Purification and reconstitution of the components of the β-adrenergic receptor-stimulated adenylate cyclase system may help to define in precise molecular terms the differences between agonists and antagonists and the functional results of the interactions of these ligands with β-adrenergic receptors.

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