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COMMENTARY

EVOLVING CONCEPTS OF PARTIAL AGONISM

THE β-ADRENERGIC RECEPTOR AS A PARADIGM

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CONCEPTS OF AGONIST- AND PARTIAL AGONIST-INDUCED RECEPTOR ACTIVATION

An agonist is defined as a compound that interacts with a neurotransmitter or hormone receptor and elicits a biologic response. A classical, competitive antagonist is a molecule that binds to a receptor, does not elicit a response, and antagonizes or prevents the binding of agonists to the receptor. A third class of receptor ligands typically binds with high affinity to receptors and, like a competitive antagonist, blocks agonist stimulation of the receptor, but unlike an antagonist is also able to elicit activation of the receptor. These latter compounds are partial agonists. Whether partial agonists partially activate all receptors in a system, or whether they activate a smaller proportion of receptors than do full agonists, remains to be answered.

Partial agonists are used for the treatment of a variety of disorders. Examples include muscarinic cholinergic partial agonists for the treatment of glaucoma, opiate receptor partial agonists in treatment of pain and β-adrenergic partial agonists (sometimes termed antagonists with intrinsic sympathomimetic activity, ISA) for the treatment of hypertension and congestive heart failure. Advantages of partial agonists in the latter settings are blockade of response to ambient levels of neurotransmitter and the absence of withdrawal syndromes or other side-effects associated with the use of antagonists [1].

We describe here historical concepts regarding partial agonism and build upon these ideas utilizing recent data. Because of the vast knowledge which has accumulated over recent years regarding the structure and activation of the β-adrenergic receptor/G_s/adenylyl cyclase system, it is used as a model for this commentary.

HISTORICAL ASPECTS OF β-ADRENERGIC RECEPTOR PARTIAL AGONISTS

In 1958, the compound dichloroisoproterenol was identified as the first example of a β-receptor blocking drug by virtue of its blockade of epinephrine-promoted tracheal relaxation [2]. After it was noted that dichloroisoproterenol not only blocked catecholamine-induced response but that it also possessed β-receptor stimulant activity, the compound was reclassified as a partial agonist [3]. Subsequently, many β-adrenergic receptor partial agonists have been developed, a few of which are shown in Fig. 1. Certain partial agonists at β-adrenergic receptors demonstrate subtype selectivity for blockade or agonism (recently reviewed by Waller [1]).

There is some structural similarity between β-agonists and -antagonists. The substituents on the aromatic ring determine whether the compound activates or blocks the receptor [4]. For example, the catechol-hydroxyl groups of isoproterenol, a full agonist, seem to be necessary for effective multipoint attachment with the β-receptor and maximal stimulation of the receptor (Fig. 2 and Ref. 6). Most competitive antagonists, typified by propranolol, lack these essential hydroxyl moieties and therefore do not stimulate the receptor. The substituted 3-phenoxy-2-propanol compound, pindolol, has only one possible hydrogen bond acceptor in its indole ring and, thus, appears to have only one of the critical determinants for agonist stimulation of the β-adrenergic receptor. Pindolol acts as a partial agonist and elicits only very mild β-adrenergic receptor responses.

Analysis of a structure–activity relationship has not proved adequate in predicting whether compounds will be partial agonists or in revealing the precise mechanism(s) for partial agonism. One event that is involved in β-adrenergic receptor action is coupling of the receptor to the activation of the stimulatory guanine nucleotide binding proteins, G_s. Binding of agonists to the β-receptor activates the receptor which, in turn, activates the G_s protein. The activated α_s subunit of G_s stimulates the catalyst of adenylyl cyclase to enhance the production of the cellular messenger cyclic adenosine 3′,5′-monophosphate (cAMP) leading to a cell-specific response. Other effectors for β-receptor activation

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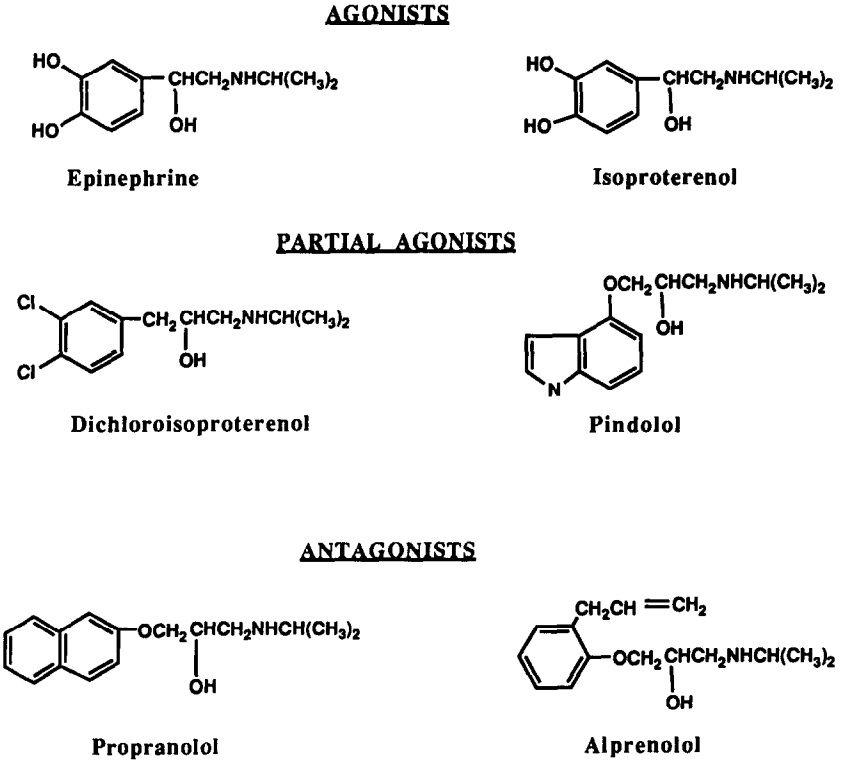

Fig. 1. Structures of some β-adrenergic receptor ligands. Antagonists have an extra oxymethylene moiety in the alkyl side chain which lengthens the distance between the charged amino group and the aromatic ring structure of the ligand.

have been suggested, but are not yet as well characterized; these include dihydropyridine-sensitive Ca^{2+} channels, sodium channels and Mg^{2+} transporters [7].

Models of receptor activation by ligands

Early studies of cholinergic agonists and partial agonists by Stephenson [8] led him to propose that the ability of agonists to promote responses is determined by both their affinity for a receptor and their intrinsic ability to stimulate a response. The latter parameter, termed efficacy (E), can be defined as the efficiency of converting ligand occupation into a stimulus (S):

$$\% \text{ maximal response} = f(S)$$

where

$$S = f[E \cdot LR/R_t].$$

Here, LR represents the ligand–receptor complex and R_t is the total number of receptors in the system. Note that efficacy depends on both the nature of the ligand as well as that of the receptor. An efficacy of zero defines an antagonist which is unable to promote a response. For some agonists of high efficacy, occupation of all receptors is not necessary to achieve a maximal response. In this case, termed receptor reserve or "spare receptors," two agonists which have different levels of fractional occupation of a given type of receptor can elicit the same maximal response. For agonists of low efficacy (i.e. partial agonists), occupation of all receptors may be necessary for maximal response; loss of receptors in such case would linearly decrease responsiveness to the partial agonist while maximal response to a full agonist may be unchanged until the receptor reserve is eliminated.

In the β-adrenergic receptor system, the efficacy of an agonist or partial agonist is typically defined in biochemical terms by the ability of a ligand to stimulate generation of the second messenger cAMP by adenylyl cyclase. For β-adrenergic receptor partial agonists, signal amplification of stimulated cAMP generation is sometimes necessary to measure this agonistic property, at least in certain cell types [9, 10]. These results imply that efficacy is a measure of G-protein activation and that differences in efficacy among partial agonists represent differing abilities of individual compounds to activate G_s.

Many studies have used physiological parameters for the determination of partial agonist activity. With the advent of molecular biology and newly developed biochemical techniques, a more molecular description of receptor activation is now possible. Here we provide a qualitative description for some of the molecular events involved in receptor activation.

Numerous models have been developed to describe the interactions of ligand and receptors [11, 12]. Two main theories have dominated thinking about this topic: occupation theory and rate theory.

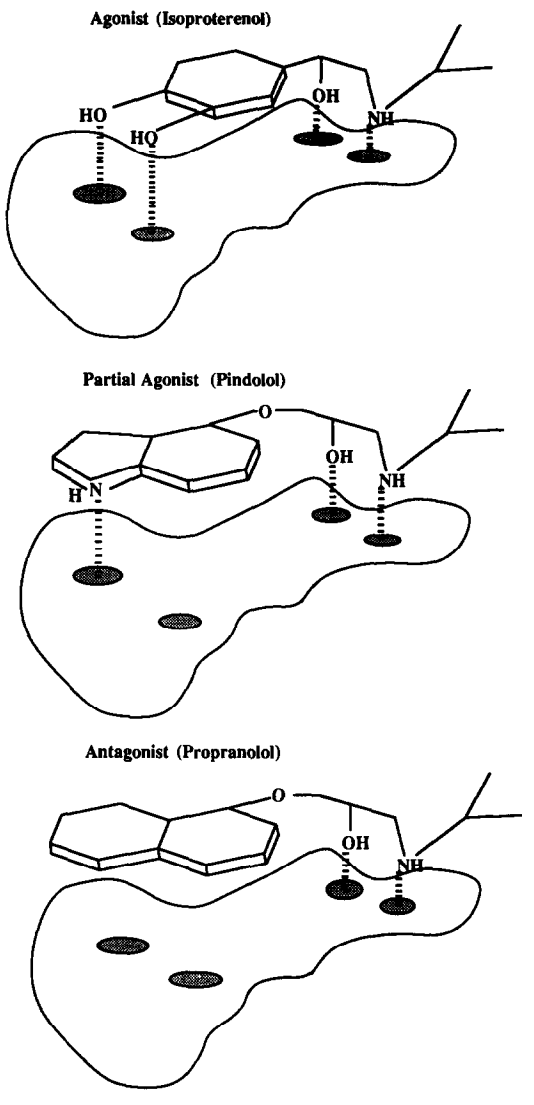

Fig. 2. β-Adrenergic receptor–ligand interaction sites. Each interaction of the ligands with the receptor requires the aliphatic hydroxyl and amino groups. The catechol-hydroxyl groups of isoproterenol interact with sites on the β-receptor (likely Ser^{204} and Ser^{207}) to elicit the conformational changes in the β-receptor necessary for activation. Pindolol has a small dipole moment for charge transfer between its indole moiety and a corresponding opposite dipole on the receptor (the naphthyl function of Phe^{290}?). Additionally, the indole nitrogen of pindolol would be capable of a hydrogen bond between itself and a hydrogen-bond acceptor or donor such as Ser^{207}, allowing partial activation of the receptor. Propranolol is an antagonist and is incapable of interacting with the receptor sites necessary for stimulation. This figure is modeled after a similar figure in Ref. 5.

(1) *Occupation theory* is based on the notion that response is a function of receptor occupancy by an agonist. Thus,

$$L + R \rightleftharpoons LR \xrightarrow{k_e} \text{response}$$

Depending on the efficacy constant k_e the amount of ligand receptor complex (LR) can be directly related to the maximal response.

(2) *Rate theory* proposes that efficacy is a function of the rate of receptor occupation by agonists. Ligands with fast rates of association and dissociation with the receptor would be considered agonists in this model, while those with slower rates would be antagonists. Accordingly, partial agonists would have intermediate rates of association and dissociation with the receptor.

Although most evidence points to the occupation theory as a better explanation for activation of receptors by agonists, some recent data support the rate theory. In particular, the studies of Stickle and Barber [13] indicate that adenylyl cyclase (and G_s) activation is proportional to the frequency of epinephrine binding to the β-adrenergic receptor of S49 lymphoma cells, i.e. the more often a receptor is activated by an agonist, the more often it can activate a G-protein molecule. Furthermore, agonists that show a lower affinity in binding to the receptor (they "unbind" more rapidly) are apparently more efficacious because the same agonist molecule can bind to and activate more receptors per unit time than would an agonist of high affinity. We, too, have found that agonists and partial agonists which stimulate intracellular cAMP accumulation to a greater extent also have, in general, lower affinity for the β-receptor (Table 1 in Ref. 10). Taken together, these data appear to challenge the dogma that ligand affinity and efficacy are independent. It might also be that the determinants of affinity, such as hydrophobicity, will also affect the interaction of a ligand with agonist- versus antagonists-specific binding sites in the receptor.

These theoretical models act as initial kinetic representations of receptor activation by agonists. A more comprehensive model has been developed to conceptualize the interactions of agonists, partial agonists and antagonists at receptors [14, 15]. This "two-state" model assumes that a receptor can pre-exist in two conformations; active (R_a) and inactive (R_i). In an equilibrium between these two states, the inactive state is prevalent when no ligand is present. An agonist activates the receptor by preferentially binding to the active conformation and thereby shifts the equilibrium toward the active receptor state:

$$\begin{array}{ccc} L + R_i & \rightleftharpoons & L\,R_i \\ \downarrow\uparrow & & \downarrow\uparrow \\ L + R_a & \rightleftharpoons & L\,R_a \end{array}$$

The relative affinity of the drug for the two conformations determines the degree of perturbation of equilibrium and consequently the magnitude of effect. A competitive antagonist would be an agent that had equal affinity for R_i and R_a and that would not alter the pre-existing equilibrium; it would antagonize a response when bound because it would inhibit the ability of an agonist to bind and alter the equilibrium. A partial agonist would bind preferentially to the "a" state, but also to the "i" state, thereby producing a partial shift in the equilibrium. This concept allows for a spectrum of possible responses depending on the relative ligand affinities for both states of the receptor.

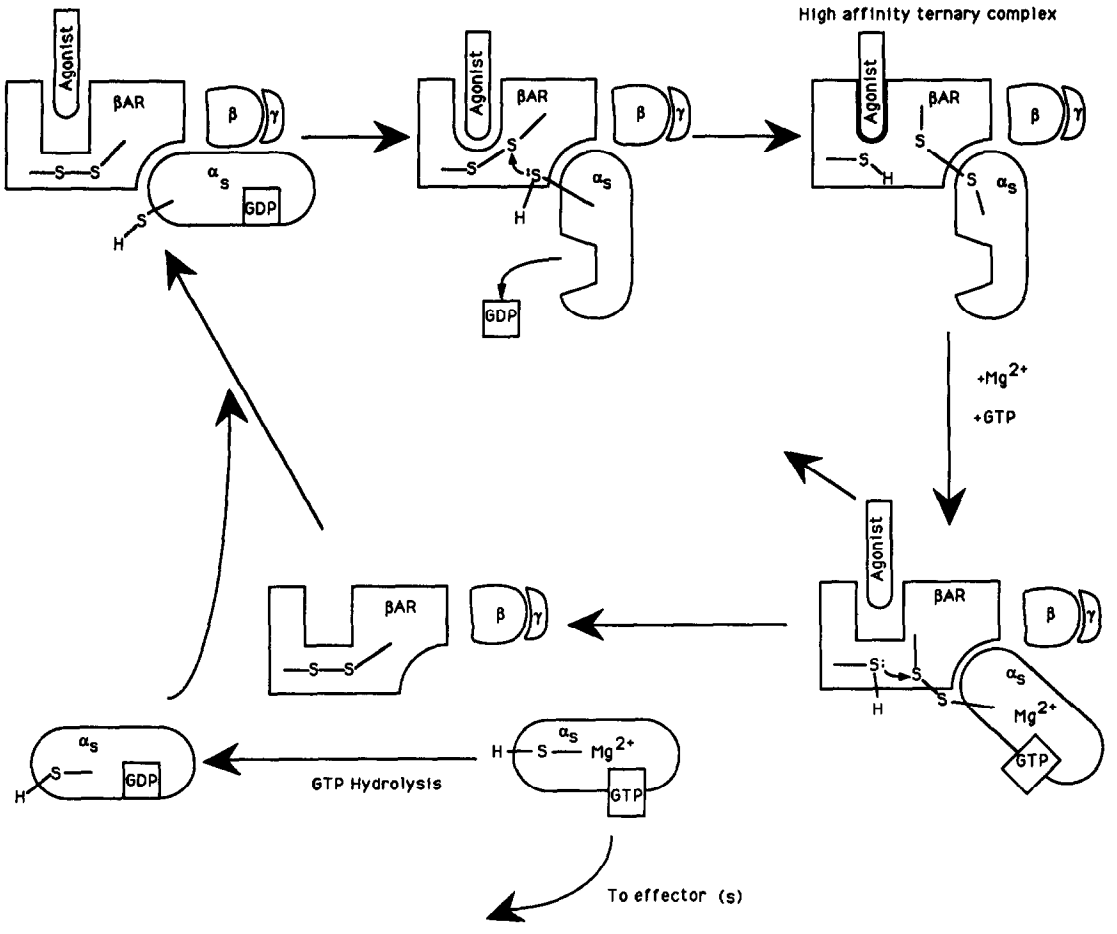

Fig. 3. Induced-fit model of β-adrenergic receptor activation by an agonist. In the unstimulated state, the β-adrenergic receptor is shown to have an internal disulfide bond between two cysteine residues, while the α subunit of G_s exhibits a free sulfhydryl group. Upon agonist binding the receptor and G_s undergo conformational changes which allow sulfhydryl exchange between the receptor and G_s and release of bound GDP. In the presence of Mg^{2+} and GTP, the G protein undergoes another conformational change to allow further sulfhydryl rearrangements and activation of the α_s subunit. The cycle is complete following GTP hydrolysis and the reassociation of the β-adrenergic receptor and G_s liganded with GDP. In this hypothetical scheme, a sulfhydryl donor from α_s has been shown; it is not known if sulfhydryl groups from the α_s-, β- or γ-subunits are important for interaction with the β-adrenergic receptor. Not shown is the role of intracellular reduced glutathione or other reductants in the sulfhydryl exchange reactions within the β-adrenergic receptor and between the β-adrenergic receptor and G_s.

Although agonists might bind to persistent conformational states of receptors, another possibility is that agonists induce these states, by analogy to the "induced-fit" hypothesis for substrate-binding to enzymes [16]. A receptor-bound agonist would induce conformational changes in the receptor protein and thereby change the affinity of the receptor for its ligand and activate the receptor to promote a cellular response (i.e. G_s-protein activation). Thus, in accordance with this notion of induced fit, the receptor would adopt the active conformation only in the presence of an agonist. In Fig. 3 we show an induced-fit model for agonist binding to a β-adrenergic receptor and the subsequent steps in G_s-protein activation (including GDP/GTP binding [guanine nucleotide exchange]) of the α subunit and dissociation of the G protein (into α_s and $\beta\gamma$) and deactivation (by GTP hydrolysis).

Thermodynamics of receptor–ligand binding

One way that has been used to distinguish agonist/antagonist action (and in turn the nature of partial agonists) has been thermodynamic analysis.

The rate of ligand–receptor association, rate of dissociation, and the fraction of receptors bound at equilibrium, are all determined by free energy changes for the system. The system always moves toward a free energy minima; thus, the lower the ΔG value for ligand interaction with its receptor, the greater the stabilization energy of the bound complex. The free energy is defined as:

$$\Delta G = \Delta H - T\Delta S$$

where ΔH, the enthalpy of interaction, is a temperature-dependent term which is related to the energy of bond-formation and bond-breaking. The entropy change, ΔS, is a measure of the change in the degree of order of all molecular structures in the system and is temperature independent. These thermodynamic measurements for analyzing the binding of ligands to receptors are determined by overall changes in ΔH and ΔS for the entire system. This system includes not only interactions between the ligand and its receptor, but also conformational changes in the receptor, changes in the mobility of the solvent, and changes in the plasma membrane (which is a very dynamic entity itself). It is important to remember that G-protein linked receptors, including β-adrenergic receptors, are membrane gylcoproteins whose interaction with membrane lipids may contribute to assessment of thermodynamic parameters.

The binding of agonists to β-adrenergic receptors is temperature dependent, while the binding of antagonists is relatively independent of temperature [17–19]. It appears that the binding of antagonists is passive and driven by thermodynamically favorable changes in entropy. Agonists, however, bind initially by a passive (entropy-driven) interaction with receptors, followed by subsequent intra- and intermolecular reactions (presumably conformational alterations and interactions with G proteins) which result in overall negative changes in enthalpy and entropy [19]. Partial agonists exhibit thermodynamic binding properties which are intermediate between agonists and antagonists; their binding is associated with an increase in entropy (like antagonists) and a decrease in enthalpy (like agonists [19]).

An alternative interpretation of the thermodynamic data described above has been proposed recently by Miklavc and colleagues [20]. Instead of passive, hydrophobic antagonist binding, these researchers maintain that the driving force of antagonist binding is determined by the favorable entropy changes incurred when the ligand moves through the opening of the receptor (in which only one-dimensional movement occurs) into a ligand-binding pocket deeper in the receptor (in which some rotational movement is allowed). The temperature-independent binding of β-adrenergic antagonists would thus be the result of loose binding of the ligand to the receptor while agonists which have more intermolecular bonds between the ligand and the receptor (e.g. hydrogen bonding of catechol hydroxyl groups to Ser^{204} and Ser^{207} of the receptor, see below) would produce larger enthalpy changes. Partial agonists, would be expected to bind loosely as antagonists part of the time and tightly as agonists the remainder of the time. This temporal variation in binding mechanism implies that the probability that a partial agonist binds to the agonist-state of the receptor would determine its intrinsic efficacy.

Full agonists typically demonstrate two interchangeable affinity states in binding to β-adrenergic receptors: one with a high-affinity-state dissociation constant (K_H), and one with a low-affinity constant (K_L) (Fig. 4). The high-affinity site appears to result from a ternary complex formed by interaction of

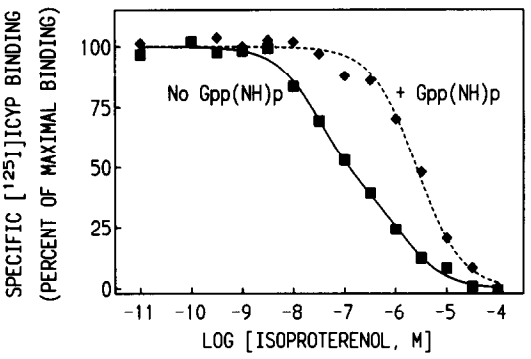

Fig. 4. Typical curve observed for competition by isoproterenol in the absence and presence of GTP for β-adrenergic receptors in cell membranes. S49 lymphoma cell membranes were incubated with various concentrations of (−)isoproterenol in the absence and presence of 100 μM GTP [^{125}I]ICYP = (−)iodocyanopindolol. The data are adapted from those shown previously in Ref. 21.

agonist, β-adrenergic receptor, and G_s, while the low-affinity site represents binding of agonist to receptors without G_s (Fig. 3). The ability of an agonist or partial agonist to stimulate adenylyl cyclase correlates closely with the amount of high-affinity state (ternary complex) formed in the presence of the agonist (detected in radioligand binding studies with membranes [22]). An even higher correlation is seen between the intrinsic activity of agonists and K_L/K_H, suggesting that the "fuller" the agonist, the better it induces or stabilizes the high-affinity state. The presumably transient existence of such "high-affinity" states in intact cells (in the presence of physiological concentrations of GTP) has been hypothesized.

A similar observation has been made for atrial muscarinic cholinergic receptors in competition studies using the agonist [^{3}H]quinuclidinyl benzilate and various cholinergic compounds: K_L/K_H is greater for full agonists compared to partial agonists, i.e. the K_L/K_H for the muscarinic agonist carbachol is about 10-fold greater than that for the partial agonist pilocarpine [23]. In reconstitution systems with muscarinic receptors and purified G_i, partial agonists were shown to induce formation of a receptor–ligand complex which has a lower affinity for its G protein, G_i (and in turn lower steady-state levels of activated G_i), compared to that formed by full agonists. These results may indicate a general mechanism for partial agonistic activity where partial agonists induce a receptor conformation having a lower affinity for its G protein than the receptor stimulated by full agonists.

Partial agonist-induced receptor phosphorylation

Further evidence for conformational changes by partial agonists have been derived from studies of agonist-promoted receptor phosphorylation. There is a strong correlation between the efficacy of β-receptor agonists in stimulation of adenylyl cyclase activity and promotion of β-receptor phosphorylation by the β-adrenergic receptor kinase (βARK; [24]).

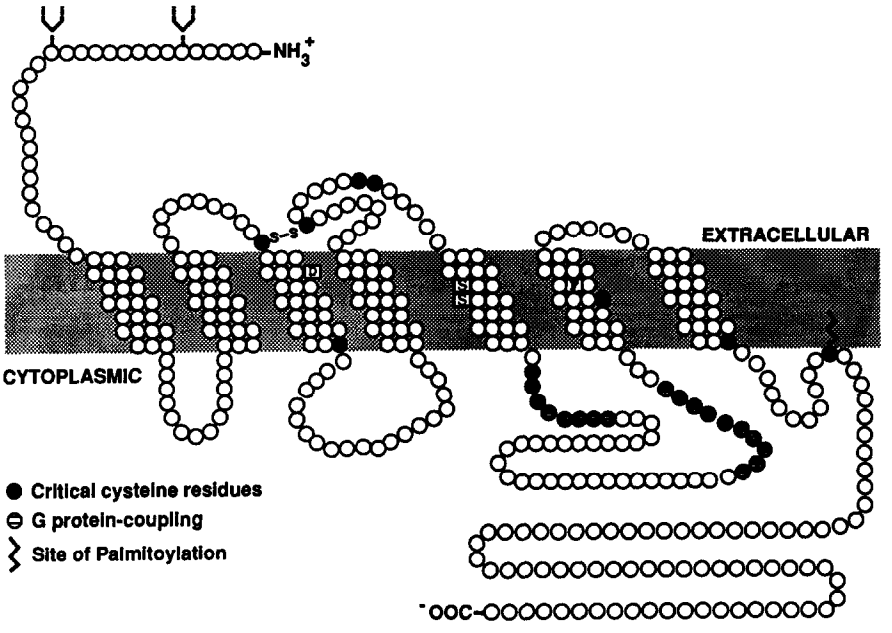

Fig. 5. General model for β-adrenergic receptor structure. The individual amino acids comprising the β-adrenergic receptor are shown as circles with extracellular NH_2 terminal and putative sites of N-linked glycosylation, seven membrane-spanning domains, and a carboxyl terminal tail. Amino acids critical for ligand binding and agonist activation of the receptor include: Asp^{113}(D) in helix III, Ser^{204} and Ser^{207}(S) in helix V, and Phe^{290}(F) in helix VI. Several cysteine residues, the site of palmitoylation, and regions involved in G protein-coupling are shown as indicated.

Even when the receptor is fully occupied, partial agonists promote only submaximal receptor phosphorylation. Antagonists and certain partial agonists (e.g. pindolol) do not stimulate receptor phosphorylation. The major difference between agonist and partial agonist-mediated phosphorylation is in the maximal velocity of βARK activity. Protein mapping of the β-receptor indicates that partial agonists show identical patterns of phosphorylation and that only the rate of ^{32}P incorporation is decreased. Thus, from these data partial agonists appear to cause the same type of conformational changes as do agonists but they do so in a smaller percentage of the receptors.

MOLECULAR STRUCTURE OF β-ADRENERGIC RECEPTORS

Recent studies have helped to elucidate many details regarding molecular architecture of the β-adrenergic receptor [25–29]. Results of these studies begin to provide more precise molecular identification of putative "efficacy sites" in the receptor. The predicted protein sequences of the β_1, β_2, and β_3, receptors are consistent with an extracellular amino terminus, three cytoplasmic and extracellular loops, seven α-helical, hydrophobic plasma membrane-spanning domains and an intracellular carboxy terminal tail (Fig. 5). Although conventionally presented in two-dimensional drawings, the hypothesized topology of the receptor suggests that the various membrane-spanning domains may interact to form a three-dimensional barrel or tube-like structure within the protein membrane (Fig. 6).

Of special interest are those domains involved in ligand binding and coupling of the receptor to the G_s protein. Binding of a ligand to a G-protein-linked receptor appears to involve multiple interactions between functional groups on the ligand and amino acid residues within the hydrophobic transmembrane core of the receptor. Residues that have been identified as crucial for high-affinity binding of agonists include: (1) two Asp residues, Asp^{113} in transmembrane helix III and Asp^{79} in transmembrane helix II (the carboxylated side chains of which have been hypothesized to serve as counter ions for the protonated amino group of the ligand), and (2) two serine residues (Ser^{204} and Ser^{207}) located in the fifth hydrophobic domain of the β-receptor, which are crucial for both agonist binding and activation of the receptor [30]. It has been hypothesized that the hydroxyl group at the *meta*-position of β-adrenergic catecholamine interacts preferentially with the side chain of Ser^{204}, while the *para*-hydroxyl group of the catecholamine interacts with Ser^{207} of the receptor [31] (Fig. 6, inset).

Molecular-modelling of the β-adrenergic receptor binding site suggests that ionic interaction between the protonated secondary amine of the ligand and the carboxylate side chain of Asp^{113} deep in the receptor binding pocket may serve to initially orient the ligand [32]. The phenyl ring of agonists (e.g. epinephrine, isoproterenol) may overlap Phe^{290} of the receptor in a hydrophobic/charge transfer

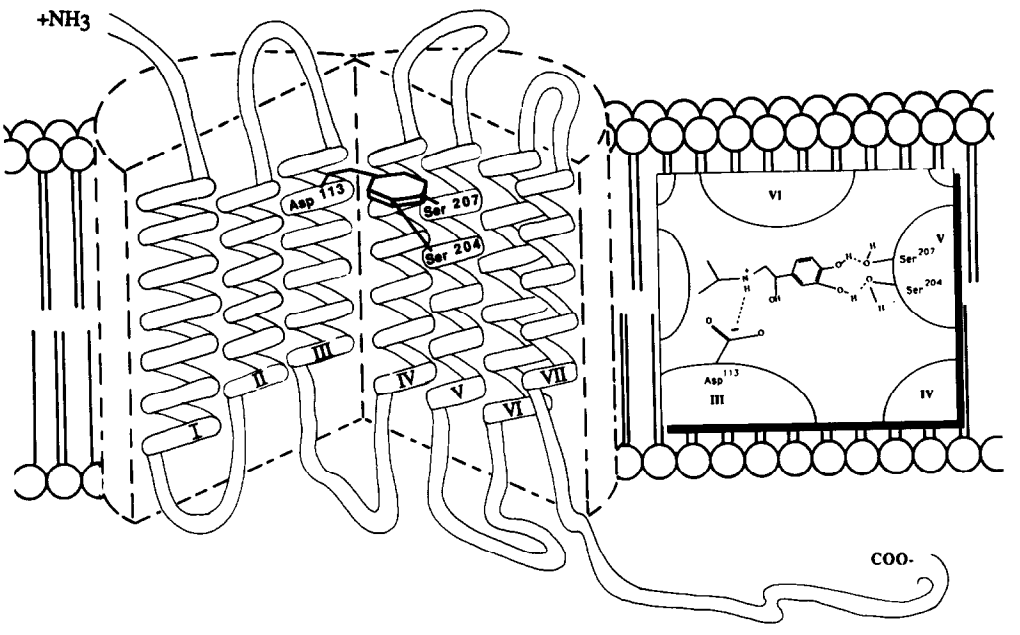

Fig. 6. Three-dimensional barrel structure of the β-adrenergic receptor. Epinephrine is shown binding inside the "barrel" composed of seven transmembrane-spanning α-helices. The critical residues for ligand binding, Asp^{113}, and Ser^{204} and Ser^{207}, are within the transmembrane-spanning region (Asp^{79} in transmembrane helix II not shown for clarity). The inset shows a view from above the receptor indicating a hypothetical orientation of epinephrine with key residues from helices III and V.

interaction [33]. Importantly, hydrogen-bonding of the agonist catechol hydroxyl moieties with Ser^{204} and Ser^{207} might induce conformational changes in the receptor required for activation of G_s (*vide infra*). Antagonists generally have an extra oxymethylene bridge which lengthens the aliphatic side chain of the molecule (Fig. 2; for review, see Ref. 34). This increased chain length probably increases the hydrophobic interactions ("ring stacking") between the aromatic ring of the antagonist aromatic ring and Phe^{290} of the receptor, thereby presumably increasing the affinity of the β-receptor for antagonists versus agonists [35]. The proposed increased "ring stacking" efficiency in antagonists versus agonists might also contribute to the observed results for thermodynamic measurements of ligand binding, i.e. antagonist binding is primarily entropy-driven (hydrophobic interactions) while entropic factors are less important in the binding of agonists.

Two ligand binding sites in the β-adrenergic receptor

The latter results, plus other lines of evidence, suggest that β-adrenergic receptors possess distinct binding sites for agonists and antagonists. For example, binding studies conducted using various ligands to interact with β_1/β_2-chimeric or α_2/β_2-adrenergic receptors indicate that the functional groups of the receptor which dictate binding of agonists are distinct from those of antagonists [36, 37]. Hence, the binding pocket formed by the seven transmembrane helices of the receptor may interact differently with ligands of varying structure.

Moreover, replacement of Asp^{113} with glutamate decreases the affinity of the receptor for agonists and antagonists [30]. The intriguing result from this work is that certain "antagonist" ligands (alprenolol and pindolol) act as partial agonists at the Glu^{113} mutant receptor. In the Glu^{113} receptor, alprenolol and pindolol promote a significant increase in adenylyl cyclase activity but this is not observed in the wild-type receptor (note, however, these compounds can promote activation of adenylyl cyclase in other systems [see Refs. 9 and 10]. Distinct agonist- and antagonist-specific binding sites might therefore exist for the aromatic moieties of at least certain ligands. The substitution of Glu for Asp in the mutant receptor apparently repositions the aromatic rings of these compounds such that they occupy the agonist-specific site of the β-receptor. Thus, partial agonism would appear to be a consequence of receptor structure as well as ligand chemistry and changes in either one can exert effects on efficacy.

β-ADRENERGIC RECEPTOR INTERACTION WITH THE G_s PROTEIN

Using α_2/β_2-adrenergic receptor chimeras, Kobilka and colleagues [36] have determined that the fifth and sixth transmembrane domains and the third cytoplasmic loop of these receptors are responsible for conferring G_s-coupling specificity. Proximal portions of the carboxyl terminus tail may also be involved in G-protein coupling, in part related to a putative fourth cytoplasmic loop formed by palmitoylation of Cys in this region [38]. Mutational

alteration of β-receptors indicates that G_s interaction requires residues at the N-terminal position of the third intracellular loop of the receptor; this region is predicted to form a cytoplasmic amphipathic α-helix [39, 40]. Hausdorff and colleagues [41] have demonstrated that the carboxyl terminal region of the third intracellular loop in the β_2-receptor is also important for β-receptor/G_s coupling. Thus, if agonist interaction with the β-receptor involves hydrogen-bonding with specific serine residues in the fifth membrane-spanning domain, conformational changes in this transmembrane helix might be translated to the bottom of the helix and might mediate interaction of the receptor with G_s. Because antagonists lack the hydrogen bond-accepting groups necessary to interact with Ser^{204} and Ser^{207} of the fifth transmembrane helix, these compounds would therefore be incapable of activating G_s via such conformational changes [42] (Fig. 6).

Role of disulfide bonds in β-adrenergic receptor function: A sulfhydryl rearrangement model for agonism

The β-adrenergic receptor contains several conserved cysteine residues which are important for G-protein linkage as well as maintaining the three-dimensional structure of the receptor [43–47]. In particular, cysteine residues 106, 184, 190 and 191 on the extracellular surface of the receptor seem to be necessary for ligand binding [47], mutation of Cys^{327} in transmembrane region VII of the receptor reduces agonist efficacy [38], and Cys^{116} and Cys^{285} in transmembrane regions III and VI, respectively, are important for agonist efficacy [45, 47]. Substitution of Ser for Cys^{285} is especially important because it decreases agonist efficacy while not greatly affecting ligand binding. Moreover, treatment of β-adrenergic receptors with sulfhydryl reducing agents (e.g. dithiothreitol) functionally activates the receptor in the absence of agonist and acts synergistically to activate receptors in the presence of agonist [48, 49]. Higher concentrations of dithiothreitol reduce the high-affinity binding of agonists to β-receptors and lead to functional deactivation [50]. Disulfide reduction by dithiothreitol also appears to unfold the receptor and affects its migration properties, i.e. the apparent molecular weight of the β-receptor shifts from M_r = 55,000 to M_r = 65,000 when the receptor is reduced by dithiothreitol [51]. Importantly, incubation with agonists mimics disulfide reduction because this incubation also leads to a prevalence of the higher molecular weight form of the receptor [52]. Furthermore, the β-adrenergic receptor is alkylated at free sulfhydryl groups by *N*-ethylmaleimide (NEM) in the presence of agonist but not by antagonists, in the absence of ligand, nor in the absence of a functional G_s [53]. Notably, alkylation of the receptor by NEM in the presence of various β-ligands correlates with their ability to stimulate adenylyl cyclase activity [54, 55]. Taken together, these data strongly suggest that agonist activation of the β-adrenergic receptor in the presence of G_s appears to cause disulfide rearrangement within the receptor and exposes free sulfhydryl groups to alkylation. An alternative notion would be that disulfide bond formation intermolecularly between receptors could lead to dimerization, which might be important for activation of G_s [45].

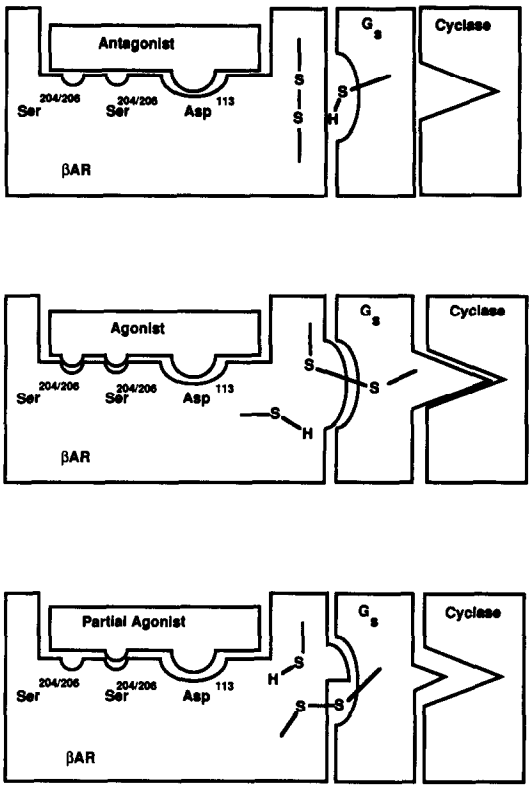

Fig. 7. Schematic model for antagonist, agonist, and partial agonist binding to a β-adrenergic receptor. Receptor, G_s, and adenylyl cyclase are shown. Key amino acid residues in the receptor involved in binding and hypothesized sites of sulfhydryl groups and disulfide bond formation within receptors and between G_s and receptors are indicated.

These results can be applied to the two-state model for agonist- and partial agonist-stimulation of the β-adrenergic receptor and adenylyl cyclase activity (Figs. 3 and 7). A full agonist would bind preferentially to the active state of the receptor and increase the probability of the receptor being in the active state at any given time. Reversible sulfhydryl rearrangement in the receptor and possibly G_s would stabilize the receptor and G_s as a high-affinity ternary complex, perhaps required to promote functional activation. According to this formulation, a partial agonist with a lower propensity toward binding and stabilizing the active state of the receptor would cause the receptor to spend correspondingly less time in this state, and full rearrangement of receptor disulfide bonds would be less likely. Because receptors are catalytic and are each able to activate many molecules of G_s [56, 57], it is attractive to propose that the β-adrenergic receptor can remain activated for a relatively long time. Disulfides may hold the receptor in this active state and promote the activation of multiple G_s molecules. G_s might act in a reciprocal manner to reduce disulfide bonds of the β-adrenergic receptor, and thereby allow the receptor to return to the inactive state.

This model would also apply if agonists and partial agonists induce conformational changes in the β-receptor (as opposed to the two-state model proposed above). Assume that one of the G_s subunits (but probably α_s) contains a disulfide or free sulfhydryl residue which would be near to a sulfhydryl residue on the β-receptor. If agonist and partial agonist binding to the receptor induces conformational changes in the receptor protein which are translated to the G_s protein, cysteine residues of either or both proteins may become exposed to cytosolic glutathione which could reduce the disulfide bonds. The movement might also translate the cysteine residues of each protein into closer proximity for interaction or might yield a subtle change in the angle between the sulfur nucleophile and that of the leaving group atom, thereby facilitating the exchange across atoms to alter nucleophile trajectory. A decreased transition-state energy (even slight) would promote disulfide rearrangement (intra- or intermolecular) [58]. Binding of GTP to α_s facilitated by the receptor agonist in the presence of Mg^{2+} would activate the G protein [7, 59], induce conformational change in the G protein, and perhaps promote another disulfide exchange reaction in order to reverse the previous exchange(s) and complete the cycle of receptor activation and inactivation (Fig. 3).

This hypothesis is supported indirectly by immunoblots of cell membranes treated with low concentrations of dithiothreitol which reveal multiple apparent molecular weight species, suggesting that more than one disulfide is being cleaved [51]. These data imply multiple disulfide rearrangements and a variety of receptor conformations. Agonists and partial agonists might then activate the receptor to varying degrees leading to different conformations stabilized by different disulfide bonds, each having different degrees of interaction with the G_s protein.

Peterson and Gerrard [60] have correlated the intrinsic efficacy of β-adrenergic agonists and partial agonists with their ability to reduce the disulfide bond of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Antagonists are unable to reduce DTNB, suggesting a direct role for the agonist molecule in altering the covalent bonds of the receptor. Whether this correlation between reducing potential and agonistic activity of β-adrenergic agonists is coincidental or mechanistically important remains to be seen. It seems more likely that the reductive potential of the catecholamine ligands is a measure of their charge-transfer ability from their electron-rich aromatic rings to an electron-deficient moiety on the β-receptor for a more efficient interaction between the pi electron ring system of these molecules.

Specificity and kinetics of G-protein stimulation of effectors

Although our bias in this commentary is toward the sulfhydryl exchange mechanism just described, there are some alternative ways that might explain (or perhaps contribute to) action of partial agonists and certain of these ideas are supported by other recent data. Several years ago Rodbell [61] proposed that G proteins are programmable messengers. This theory describes G_α subunits as soluble proteins which might be released from the plasma membrane upon hormone receptor binding. Modification of the α subunits in the cytosol by kinases, methylases, and proteases would lead to a different regulatory structure that might be considered "programmable." This theory provided a way in which agonists could promote multiple and varied cellular responses. At the time of Rodbell's proposal, only weak evidence existed that G_α subunits could be released from the membrane [62]. In more recent studies, Milligan and Unson [63] have shown release of α_s from the plasma membrane of C6 glioma cells by Gpp(NH)p and we have found that receptor agonists can induce the release of α_s from cell membranes of S49 lymphoma cells [57, 64]. In theory this α_s might stimulate intracellular effectors other than adenylyl cyclase. It is interesting to speculate that partial agonists might stimulate the G_s protein to varying degrees, and that the released α_s would alter cellular response perhaps via regulating effector molecules to differing extents than would full agonists.

Another possibility for a mechanism of partial agonists is linkage of activated receptors to G proteins with opposing action. Although controversial,* some evidence suggests that the β-adrenergic receptor, for example, can interact not only with the G_s protein but also with G_i [65, 66]. Kenakin and Morgan [67] recently presented a model which describes the kinetics of agonists interacting with a "promiscuous" receptor. Here, the relative amounts of each G protein in a tissue would determine agonist potency for a response. Ligands which interact somewhat differently with the β-receptor (agonists vs partial agonists) might exhibit varied levels of activation of G_s versus G_i, and thereby different physiologic responses in different tissues. Determinants of interactions of receptors with different G proteins have not been defined but should be a fruitful area for future research.

SUMMARY AND CONCLUSIONS

The exact mechanism of receptor activation at the molecular level are still not known, nor do we completely understand the precise factors that distinguish agonist- and partial agonist-induced activation. Nevertheless, recent years have brought forth an explosion of new information regarding β-adrenergic receptor structure and ligand-induced activation. Partial agonists are likely intermediate in their ability to interact with crucial serine residues (Ser^{204} and Ser^{207}) on the β-adrenergic receptor; these interactions allow either incomplete stimulation of the entire receptor population, or full stimulation of only a portion of the entire receptor population. From the work presented by Tota and Schimerlik [23] for the muscarinic cholinergic receptor (another G-protein coupled receptor), it is likely that partial agonists induce or stabilize receptor conformations that have a lower affinity for their G protein compared to receptors stimulated by a full agonist.

Molecular cloning of β-adrenergic receptors and

* Insel PA and Koachman AM, Do beta-adrenergic receptors in intact cells couple to G_i, the inhibitory guanine nucleotide binding protein of adenylate cyclase? *Endocrine Soc. Program and Abstracts*, p. 229, 1985.

analyses of mutated and chimeric receptors expressed in transfected systems have indicated that domains of the receptor that bind agonists may be different from those with which antagonists interact. Thus, the ability of a partial agonist to interact with these two different domains may be a determinant of efficacy.

Agonists alter the sulfhydryl redox status of the β-adrenergic receptors in the presence of G_s. Disulfide rearrangement has been postulated to provide a structural constraint which biases G-protein-linked receptors in the "ground state" [68] and may be important for stabilizing the active state of the receptor and holding the agonist/receptor/G_s ternary complex in the high-affinity state. Partial agonists induce this state less efficaciously or are less capable of holding the receptor in the active conformation to allow disulfide exchange to take place. The extent of receptor stimulation may dictate which G proteins are activated by a particular receptor, and thus which cellular effectors are stimulated. Alternatively, the level of activation of a receptor may translate into varying states of activation of a particular G protein (stabilized in part by disulfide bonds). Techniques such as fluorescence energy transfer in reconstitution systems or nuclear magnetic resonance spectroscopy should prove useful in distinguishing among these possible mechanisms. Ultimately, as a long-term goal, X-ray crystallography of unoccupied receptors and receptors liganded by partial or full agonists may provide definitive insights. Although definitive answers are not yet possible, the rapid progress in understanding aspects of receptor structure allows a reformulation of ideas regarding the molecular basis of efficacy and partial agonism. Continuing efforts will almost certainly focus on the role of domains of receptor proteins and specific amino acid residues within those domains. Our model for disulfide rearrangement may provide a useful, testable hypothesis for further studies.

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