Journal of Supramolecular Structure 8:501–510 (1978)
Transmembrane Signaling 75–84

Beta-Adrenergic Receptors: Regulatory Role of Agonists

Robert J. Lefkowitz, Lee E. Limbird, Lewis T. Williams, and Michael Wessels

Duke University Medical Center, Durham, North Carolina 27710

Direct radioligand binding studies have been used to probe the molecular mechanisms whereby agonist catecholamines regulate the function of beta-adrenergic receptors in a model system, the frog erythrocyte. The unique characteristics of agonist as opposed to antagonist action are first, the ability to stimulate the adenylate cyclase through the receptor and second, the ability to desensitize the system by alterations induced in beta-adrenergic receptors. These properties of agonist are not shared by antagonist despite the high affinity and specificity of antagonist binding to the beta-adrenergic receptors. Agonist and antagonist receptor complexes may be distinguished in a variety of ways including differences in their sensitivity to regulatory guanine nucleotides and also by gel chromatography on AcA 34 Ultragel. The agonist receptor complex appears to elute from the columns with an apparently increased size. A "dynamic receptor affinity model" of beta-adrenergic receptor action is proposed which features several distinct conformational states of the receptor. Agonists have much higher affinity for the physiologically active or coupled state of the receptor, whereas antagonists have equal affinity for both. In addition, a third "desensitized" state of the receptor is also postulated to exist.

Key words: receptor, catecholamines, agonist, adenylate cyclase, erythrocyte

Studies aimed at elucidating the molecular mechanisms by which agonist hormones and drugs stimulate biological processes such as adenylate cyclase have received a great impetus over the past several years from the development of specific radioactively labeled ligands which can be used to study the receptors directly. Much interest has focused on the receptors through which catecholamines stimulate adenylate cyclase which are known as the beta-adrenergic receptors. In contrast with most other adenylate cyclase-coupled receptors for which only agonist ligands are available (eg, glucagon, vasopressin, parathyroid hormone, adrenocorticotropic hormone [ACTH], etc), for the beta-adrenergic receptors both radiolabeled antagonist and agonist ligands are available. Recent studies in our laboratory using such radioligands have shed new light on the mechanisms by which

Received for publication April 4, 1978; accepted June 1, 1978.

0091-7419/78/0804-0501$02.00

hormone agonists interact with and regulate adenylate cyclase systems through specific receptors. We are trying to understand the two unique characteristics of agonist as opposed to antagonist action. These are the ability to stimulate the enzyme adenylate cyclase on the one hand and to desensitize the system to further stimulation by agonists on the other.

METHODS

All the studies presented in this communication were performed using membrane fractions derived from erythrocytes from southern grass frogs. Methods for the preparation of the membranes [1, 2] for the adenylate cyclase assays [3] and for radioligand binding studies have been previously published [4, 5]. Radioligands used were (–)[^{3}H]dihydroalprenolol, a potent beta-adrenergic antagonist with a specific radioactivity of 33–58 Ci/nmole, and (±) [^{3}H]hydroxybenzylisoproterenol, an agonist with specific radioactivity of 10–26 Ci/mmole. The sources of all the reagents were as previously described [1–5].

RESULTS

Guanine Nucleotides

In frog erythrocyte membranes, as in many other systems, hormonal stimulation of adenylate cyclase demonstrates marked dependence on the presence of guanine nucleotide [6, 7]. This finding is illustrated in Figure 1. Adenylate cyclase assays were performed at a low substrate adenosine triphosphate (ATP) concentration, 0.12 mM. At this concentration very little stimulation of the enzyme by the beta-adrenergic agonist isoproterenol could be observed whereas stimulation by fluoride ion was quite substantial. However, when guanosine triphosphate (GTP) was added, it had little effect on basal or fluoride-stimulated enzyme activity, whereas isoproterenol-stimulated activity was markedly potentiated.

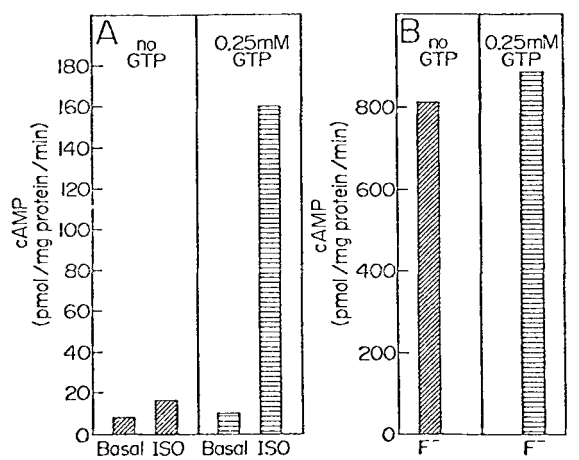


Fig 1. GTP requirement for isoproterenol stimulation of adenylate cyclase in frog erythrocyte membranes. A crude particulate fraction was used for these experiments. When present the concentration of isoproterenol was 0.1 mM, and that of NaF 10 mM. Figures 1 and 2 from [6], reprinted with permission.

The ability of guanine nucleotides such as GTP or its nonhydrolyzable analog Gpp(NH)p to potentiate catecholamine stimulation of adenylate cyclase is one of two major effects which the nucleotides have on this hormone-sensitive system. The other effect of guanine nucleotides is a unique agonist-specific effect on binding affinity [8, 9]. Figure 2 demonstrates the effect of the guanine nucleotide analog Gpp(NH)p on the kinetics of [^{3}H] hydroxybenzylisoproterenol (agonist) binding to the beta-adrenergic receptors. In the absence of guanine nucleotide, agonist binding is only very slowly dissociable. However, when guanine nucleotide is added, binding becomes very rapidly reversible (Fig 2). By contrast, antagonist [^{3}H] dihydroalprenolol binding shows rapid association and dissociation and the kinetics of (−)[^{3}H] dihydroalprenolol binding are not affected by guanine nucleotides (data not shown) [6]. Thus, in addition to their requirement for beta-adrenergic receptor-mediated stimulation of adenylate cyclase, the guanine nucleotides also cause a unique agonist-specific reduction in ligand binding affinity to the beta-receptors.

Solubilized Preparations

In an attempt to study the beta-adrenergic receptors in molecular terms we turned to the use of solubilized receptor preparations. Previously we have demonstrated that the beta-adrenergic receptors in frog erythrocyte membranes could be solubilized in high yield with the plant glycoside digitonin [2]. We have performed experiments in which beta-adrenergic receptors were labeled either in membranes or in soluble preparations with the agonist [^{3}H] hydroxybenzylisoproterenol or the antagonist [^{3}H] dihydroalprenolol [10]. Figure 3 summarizes some of these results. A major finding is that when receptors are prelabeled in membranes with [^{3}H] hydroxybenzylisoproterenol followed by solubilization (middle panel), the receptors appeared to chromatograph with a larger apparent molecular size on AcA 34 ultragel columns than when they were prelabeled with antagonists such as [^{3}H] dihydroalprenolol (lower panel). The apparent beta-adrenergic receptor size as judged by antagonist binding was the same regardless of whether the antagonist was used

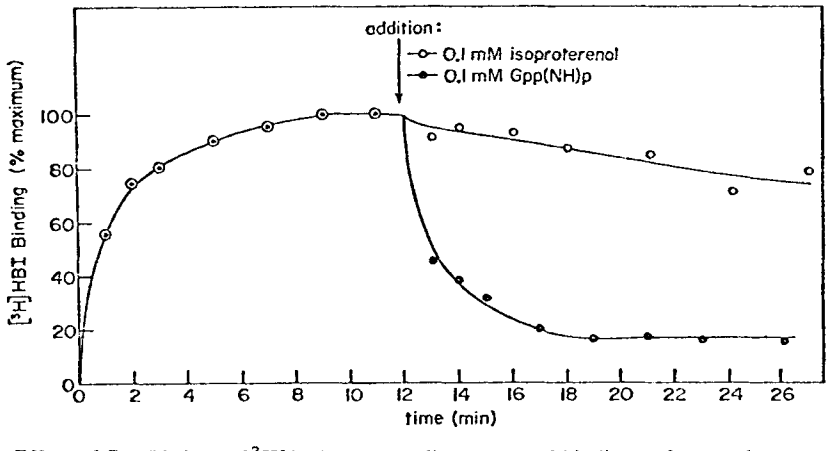


Fig 2. Effect of Gpp(NH)p on [^{3}H] hydroxybenzylisoproterenol binding to frog erythrocyte membranes. Purified membranes were used.

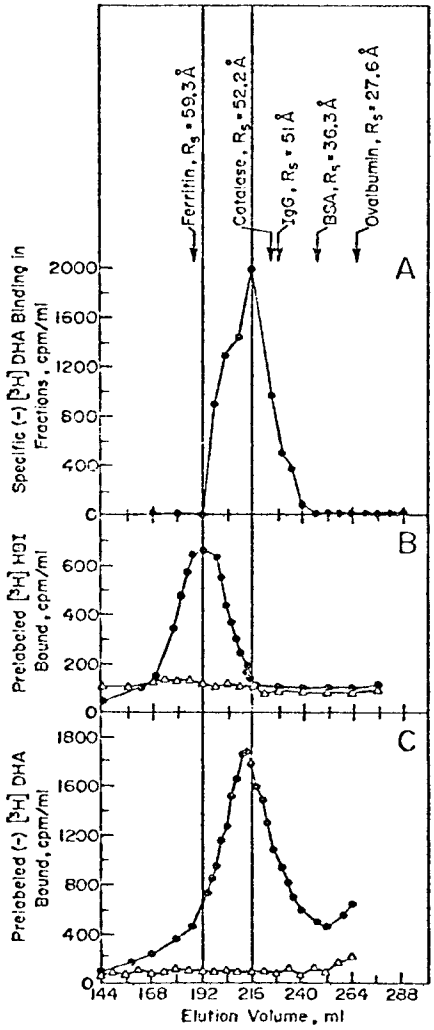


Fig 3. Gel filtration of digitonin-solubilized beta-adrenergic receptors on AcA 34 ultragel. A) Elution profile of beta-adrenergic receptor binding activity solubilized from control frog erythrocyte membranes (the data plotted represent [^{3}H] dihydroalprenolol binding detected in the eluted fractions). B) Elution profile of agonist [^{3}H] hydroxybenzylisoproterenol receptor complex (•). [^{3}H] Hydroxybenzylisoproterenol was used to prelabel the receptors in the membranes prior to solubilization with digitonin and chromatography on the AcA 34 column. An assessment of "nonspecific" [^{3}H] hydroxybenzylisoproterenol binding was made by chromatographing solubilized material derived from membranes labeled with [^{3}H] hydroxybenzylisoproterenol in the presence of 10 μM propranolol (△). C) Elution profile of antagonist [^{3}H] dihydroalprenolol receptor complex (•). Protocol similar to B except that prior to solubilization [^{3}H] dihydroalprenolol was used to prelabel the receptors in the membranes. "Nonspecific" binding was assessed by chromatographing material solubilized from membranes prelabeled with [^{3}H] dihydroalprenolol in the presence of 0.1 mM isoproterenol (△). From [10], reprinted with permission.

to prelabel receptors in the membranes or was used to study binding in the column fractions obtained from the gel filtration columns (upper panel). The larger apparent molecular size of the receptor labeled with agonist is not due to a physical association of the receptor with the adenylate cyclase enzyme, since the enzyme was found to elute in separate fractions (not shown) [10].

A detailed explanation in biochemical terms of the apparent increase in molecular size of the agonist-labeled receptor is not available at present. This could represent agonist-induced association of the beta-adrenergic receptors with other beta-adrenergic receptors or other components of the catecholamine-sensitive adenylate cyclase system. As noted above, the increased receptor size appears not to represent a physical association with the enzyme adenylate cyclase. An additional explanation for the increased apparent receptor size is that it may represent angonist-induced conformational alteration of the receptor to a more asymetric form – for example, a lengthening of the transmembrane axis of the receptor.

Desensitization of the Beta-Adrenergic Receptor-Coupled Adenylate Cyclase

In addition to their ability to stimulate the adenylate cyclase, beta-adrenergic catecholamines also can desensitize the enzyme to further stimulation by catecholamines in certain systems [11–17]. It should be appreciated from the outset of this discussion 1) that not all systems sensitive to beta-adrenergic stimulation show this effect, and 2) that there appear to be a number of different forms of such refractoriness having distinct and possibly overlapping mechanisms. Perkins and associates [14, 15] made an important contribution in this area by clearly delineating that there are at least two very distinct mechanisms which may be involved in the development of refractoriness to catecholamines. One mechanism, which they have termed "heterologous" desensitization, [14, 15], is probably only seen in intact cells. In heterologous desensitization a cell which has been exposed to any one of a number of hormonal stimulators of adenylate cyclase such as prostaglandins or catecholamines, develops refractoriness to further stimulation of cyclic AMP (adenosine monophosphate) levels by all hormonal stimulators. Thus this effect is not receptor-specific. The effect appears to largely disappear if adenylate cyclase activity is investigated in broken-cell preparations. A distinct form of refractoriness which is receptor-specific is called "homologous" desensitization. This form of desensitization persists in broken-cell preparations from desensitized cells [15]. A cell which has been desensitized by previous exposure to beta-adrenergic agonist will show refractoriness only and specifically to subsequent stimulation by beta-adrenergic agents. Stimulation by another hormonal activator such as prostaglandin E_1 appears normal. Conversely, prostaglandins may desensitize to subsequent prostaglandin stimulation, whereas stimulation by catecholamines is then normal. We have been particularly concerned with elucidating the mechanisms for catecholamine-induced specific or "homologous" desensitization in the model system of frog erythrocytes. Prior exposure of these cells to either catecholamines [11, 12, 18, 19] or prostaglandins [20] for several hours is followed by specific desensitization to subsequent stimulation by the hormonal activator to which the cells have been previously exposed. Several years ago we made the observation that specific desensitization of the adenylate cyclase by catecholamines was consistently associated with a fall in the number of functional beta-adrenergic receptors that could be measured by binding of the radiolabeled beta-adrenergic antagonist (–)[^{3}H]dihydroalprenolol [11, 21]. It should be stressed that stimulation of the enzyme by prostaglandin E_1 or fluoride is entirely normal. Only stimulation by beta-adrenergic catecholamine agonists is decreased.

Another consistent finding has been that although the fall in beta-adrenergic receptor number parallels the decline in catecholamine-responsive cyclase in many respects (including specificity, temperature dependence, etc), the extent of the fall in receptor number is in general less than the fall in catecholamine-stimulated adenylate cyclase. In some systems studied by other investigators (S49 lymphoma cell [13] or astrocytoma cells [15]) although a fall in beta-adrenergic receptor number as assessed by [^{125}I]hydroxybenzyl-

pindolol binding occurs in association with desensitazation, the discrepancy between fall in receptor number and fall in enzyme activity is even more striking than has been the case in the frog erythrocyte. In an attempt to gain further insight into the mechanisms of desensitization and the possible changes in receptors which might be contributing to this desensitization, we performed binding studies on membranes prepared from control and desensitized cells using both the radiolabeled agonist [^{3}H] hydroxybenzylisoproterenol and the antagonist [^{3}H] dihydroalprenolol. The pertinent finding is presented in Figure 4. It can be observed that there is an excellent quantitative agreement between the percentage fall in agonist binding and the percentage fall in catecholamine-stimulated enzyme activity over a period of many hours of desensitization [21]. By contrast, there was significantly less percentage reduction in [^{3}H] dihydroalprenolol binding. Thus, binding of the agonist [^{3}H] hydroxybenzylisoproterenol appears to reflect even more closely the desensitization of hormone-responsive adenylate cyclase than does antagonist binding.

Another point of some interest relates to the question of whether the desensitized state of the receptor is one which is associated with high-affinity occupancy of the receptors by radiolabeled agonist [22]. The data in Figure 5 suggest that this is not the case. In these experiments, intact frog erythrocytes were desensitized by 2.5 h incubation with 10^{-7}M [^{3}H] hydroxybenzylisoproterenol agonist. The cells were then extensively washed and lysed, and purified membranes were prepared. After extensive washing, the membranes were assayed for [^{3}H] dihydroalprenolol binding. In addition separate aliquots of the membranes were counted directly to determine the amount of [^{3}H] hydroxybenzylisoproterenol bound. [^{3}H] Hydroxybenzylisoproterenol bound was determined as that specifically bound which represented the difference between [^{3}H] hydroxybenzylisoproterenol bound to membranes from cells preincubated with [^{3}H] hydroxybenzylisoproterenol alone as compared with that from cells preincubated with [^{3}H] hydroxybenzylisoproterenol in the presence of propranolol. It can be seen from Figure 5, that the amount of [^{3}H] hydroxybenzylisoproterenol residually bound to membranes from the desensitized

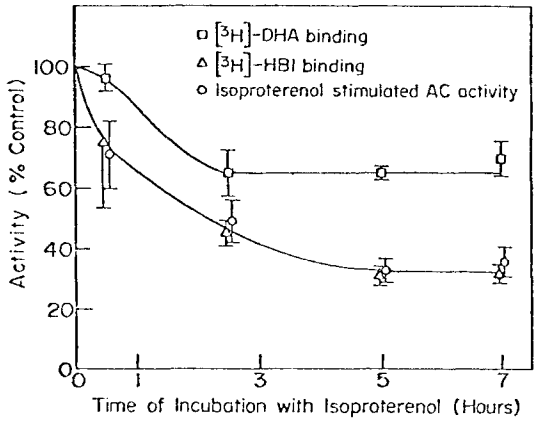


Fig 4. Differences between agonist and antagonist binding to frog erythrocyte membranes following desensitization of whole cells. Intact frog erythrocytes were desensitized by preincubation with 0.1 mM isoproterenol for the indicated time periods. Binding was assayed in washed particulate fractions from control and desensitized cells at ligand concentrations of 5–10 nM. Differences between percentage fall in [^{3}H] dihydroalprenolol and [^{3}H] hydroxybenzylisoproterenol binding and between percentage fall in [^{3}H] dihydroalprenolol binding and percentage fall in isoproterenol-stimulated adenylate cyclase were significant, $p < 0.05$ or greater at all time points except 0.5 h. Differences between percentage fall in [^{3}H] hydroxybenzylisoproterenol binding and percentage fall in adenylate cyclase activity were not significant at any time point. From [21], reprinted with permission.

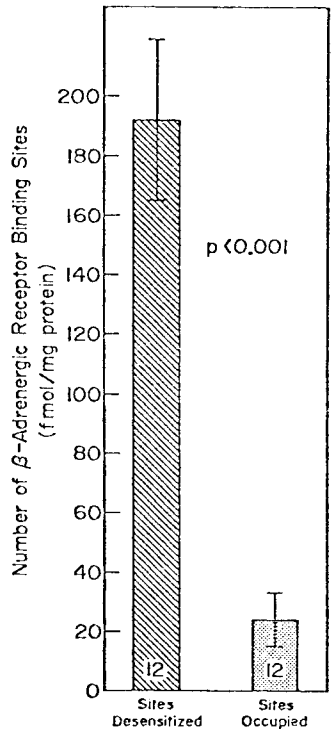
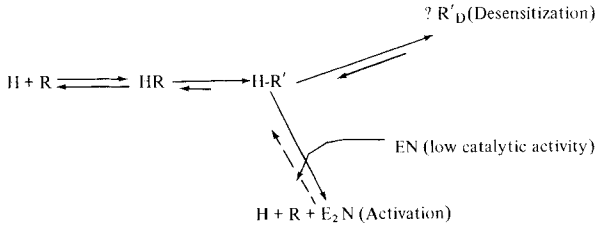


Fig 5. Relation between tightly bound [^{3}H]hydroxybenzylisoproterenol and desensitization of beta-adrenergic receptors. Frog erythrocytes were desensitized by incubation with 10^{-7}M [^{3}H]hydroxybenzylisoproterenol for 2.5 h. "Sites desensitized" refers to the fall in [^{3}H]dihydroalprenolol binding to washed particulate fractions from these cells, assayed at saturating [^{3}H]dihydroalprenolol concentrations. "Sites occupied" refers to the residual tightly bound [^{3}H]hydroxybenzylisoproterenol specifically bound to these washed particulate fractions. From [22], reprinted with permission.

cells does not account for the number of sites desensitized. Thus, the desensitized state of the beta-adrenergic receptors in these cells is one which is not associated with high-affinity agonist occupancy [22].

DISCUSSION

We have recently described a schema [6] which serves to summarize our current conceptualizations of the various states of the beta-adrenergic receptor. The main value of this schema is its ability to pull together a large number of observations concerning beta-adrenergic and other adenylate cyclase-coupled receptors as well as its ability to suggest further lines of experimentation.



In this schema H stands for hormone agonist such as catecholamine, R for the beta-adrenergic receptor, E the enzyme adenylate cyclase, and N regulatory guanine nucleotide. The essential feature of this schema is that the beta-adrenergic receptor can exist in several different functional states. There are two basic forms of the receptor envisaged, which are depicted as R and R′ respectively. R is the resting or inactive state, and R′ is the state induced as a result of agonist binding and is associated with activation of the enzyme adenylate cyclase. Antagonists such as dihydroalprenolol, propranolol, etc, bind to R and do not induce the formation of R′. Agonists, however, have much higher affinity for the R′ form and hence force the equilibrium toward the formation of the high-affinity binding complex. At rest in the membranes the overwhelming majority of the receptors are presumably in the R conformation. When agonists promote the formation of H-R′ a further interaction occurs between H-R′ and the adenylate cyclase system. Molecular details of this interaction remain scarce; however, one possibility is that the interaction occurs between the H-R′ form of the receptor and the nucleotide liganded form of the enzyme. Alternatively H-R′ might first interact with the enzyme E to form an intermediate state H-R-E of low catalytic activity, which then reacts with nucleotide. This interaction is associated with two concerted events. First, the enzyme adenylate cyclase is activated to a high-activity state indicated as E_2N. Second, the H-R′ high-affinity complex is dissociated into free hormone agonist and free receptor in the R conformation. The receptor is then free to recycle through the sequence again. It would appear that these R and R′ forms of the receptor can be distinguished by gel filtration chromatography under appropriate conditions as described above. Thus, the receptors solubilized after prelabeling of the membranes with [^{3}H]hydroxybenzylisoproterenol might represent the R′ form of the receptor. This is, as noted above, the guanine nucleotide-sensitive state responsible for activation of adenylate cyclase.

In our schema, antagonist would label only the R state of the receptor in the membranes, as it is the predominant state which exists in the absence of agonist. Binding of antagonist does not induce the formation of R′ and the bulk of the receptors remain in the R conformation upon binding antagonist. The state of the receptor labeled with [^{3}H]-dihydroalprenolol would be the R state. It should be noted that this schema makes no specific assignments as to the molecular mechanisms responsible for the agonist-induced transition from the R to the R′ state of the receptor.

The proposed model provides a conceptual framework for understanding many of the experimental observations on the effects of nucleotides and hormones on the beta-adrenergic receptor and adenylate cyclase. The schema incorporates alterations in both the receptor and cyclase during hormone and nucleotide activation. To reiterate the main points of our conceptualization:

1. A state of "tight" binding HR is formed prior to the increase in adenylate cyclase catalytic activity.Formation of this complex is responsible for the facilitation of the activation of the cyclase by regulatory nucleotide.

2. In the presence of the hormone-receptor complex HR′ the cyclase is potentially very reactive with nucleotide. This activation of the enzyme by nucleotide is associated with a dissociation of the tightly bound complex HR′ into free H and unoccupied R.

Although we have incorporated these two concepts into a specific reaction schema (shown above), the exact order and nature of the reactions may be modified and still be consistent with the main concepts [6].

This relatively simple schema can explain a number of the seemingly paradoxical effects of nucleotides reported in the literature [6].

1. The nucleotide analog Gpp(NH)p increases the apparent affinity of agonists as assessed by adenylate cyclase concentration-response curves in frog erythrocytes [9] and other membranes. Since the reaction of Gpp(NH)p with HR′ is rapid and since the equilibrium strongly favors the formation of H+R+(E_2N), the presence of Gpp(NH)p drives the reaction sequence towards the formation of the maximally activated state (E_2N), thereby increasing the amount of activation which even low concentrations of hormone can achieve. The net result is an increase in apparent affinity. By contrast nucleotides such as GTP, whose action is reversible, do not generally appear to increase apparent affinity of hormone stimulation.

2. The fact that Gpp(NH)p dissociates irreversibly bound agonist (HR′) but not antagonist while causing maximal irreversible activation of the cyclase in the presence of hormone presumably accounts for the ability of nucleotides to reduce the apparent affinity of agonists (but not antagonists) for beta-adrenergic receptor binding sites. This schema adequately accounts for the seemingly paradoxical observations that Gpp(NH)p increases the apparent affinity of beta-adrenergic agonists for stimulation of adenylate cyclase while reducing the apparent affinity for the receptor binding sites.

3. Nucleotides cause a reversal of the decrease in apparent beta-receptor number caused by preincubation of frog erythrocyte membranes with beta-agonists [11]. Since the apparent decrease in receptor number under these circumstances is due to formation of tightly bound receptors in the form HR′, nucleotides restore apparent receptor number to normal by dissociating the HR′ complex and forming unoccupied R available for further binding.

4. If membranes are preincubated with hormones and the nucleotide Gpp(NH)p and then washed or diluted, the cyclase is in an activated state which is partially inhibited by subsequent exposure to agonists [23, 24]. This apparent paradoxical effect of hormones would in fact be predicted if the step in which the Gpp(NH)p reacts with H-R′ is partially reversible (dotted arrow in the model). Thus, after preincubation of membranes with hormone and Gpp(NH)p followed by washing, the cyclase is in the state E_2(N) in the absence of free nucleotide or hormone. Subsequent addition of hormone would drive the reaction (dotted line) towards the (inactive) state EN or HRE and hence would partially reduce the measured catalytic activity.

5. In the presence of ATP concentrations high enough to serve as adenylate cyclase substrate, but too low to interact with the regulatory nucleotide site, agonists such as glucagon and isoproterenol do not effectively stimulate the adenylate cyclase. This would be expected since under these conditions E_2(N) could not be formed. However, upon the addition of regulatory nucleotide (GTP), agonist would promote the formation of E_2(N) and consequently stimulate catalytic activity.

In addition, it would appear necessary to postulate the existence of at least one other conformation of the receptor which is indicated in the schema as R'_D or "desensitized" conformation. This conformation binds neither agonist nor antagonist and cannot activate adenylate cyclase. Since, under the assay conditions we have used, [^{3}H]hydroxybenzylisoproterenol binding appears to label mostly, if not exclusively the R′ state, it would be expected that the fall in [^{3}H]hydroxybenzylisoproterenol binding after desensitization would very closely parallel the fall in catecholamine-responsive enzyme activity. By con-

trast, since [^{3}H] dihydroalprenolol labels all receptor of both the R and R′ type, the percentage fall in [^{3}H] dihydroalprenolol binding is less impressive. As with the activation portion of this sequence, the crucial element in desensitization maybe the H-R′ or high-affinity agonist binding form of the receptor. Thus, the H-R′ form of the receptor which is formed only by agonist and not antagonist may be viewed as a crucial branch point in the sequences leading to activation and desensitization of the adenylate cyclase. It is the presumed precursor of both pathways. At present little is known about the factors which regulate the appearance of the desensitized form of the receptors in relationship to the total pool of receptors, or the exact point in the schema distal to HR′ where it might arise.

This "dynamic receptor affinity model" [6] for explaining the various receptor transitions involved in activation and desensitization stresses the existence of several distinct forms of the receptor with varying affinities for agonist. It will be of interest to see how widely applicable this model is to other adenylate cyclase-coupled receptor systems. However, the lack of generally available receptor antagonists in several of these systems may hamper progress along these lines.

ACKNOWLEDGMENTS

This study was supported by Health Education and Welfare grants HL 16037 and HL 20339 and a grant-in-aid from the American Heart Association with funds contributed in part by the North Carolina Heart Association. Dr Lefkowitz is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

1. Mukherjee C, Caron MG, Mullikin D, Lefkowitz RJ: Molec Pharmacol 12:16, 1976.
2. Caron MG, Lefkowitz RJ: J Biol Chem 251:2374, 1976.
3. Lefkowitz RJ: J Biol Chem 249:6119, 1974.
4. Williams LT, Jarret L, Lefkowitz RJ: J Biol Chem 251:3096, 1976.
5. Lefkowitz RJ, Williams LT: Proc Natl Acad Sci USA 74:515, 1977.
6. Williams LT, Lefkowitz RJ: J Biol Chem 252:7207, 1977.
7. Rodbell M, Krans HMG, Pohl SL, Birnbaumer L: J Biol Chem 246:1872, 1971.
8. Maguire ME, Wiklund RA, Anderson HJ, Gilman AG: J Biol Chem 251:1221, 1976.
9. Lefkowitz RJ, Mullikin D, Caron MG: J Biol Chem 251:4686, 1976.
10. Limbird LE, Lefkowitz RJ: Proc Natl Acad Sci USA 75:228, 1978.
11. Mukherjee C, Lefkowitz RJ: Proc Natl Acad Sci USA 73:1494, 1976.
12. Mickey JV, Tate R Lefkowitz RJ: J Biol Chem 250:5727, 1975.
13. Shear M, Insel PA, Melmon KL, Coffino P: J Biol Chem 251:7572, 1976.
14. Su Y-F, Cubeddu L, Perkins JP: J Cyclic Nucleotide Res 2:257 1976.
15. Johnson GL, Wolfe BA, Hardin TK, Molinoff PB, Perkins JP: J Biol Chem (In press).
16. Remold O'Donnell E: J Biol Chem 249:3615, 1974.
17. DeVellis J, Brooker G: Science 186:1221, 1974.
18. Mickey JV, Tate R, Mullikin D, Lefkowitz RJ: Molec Pharmacol 12:409, 1976.
19. Mukherjee C, Caron MG, Lefkowitz RJ: Endocrinology 99:347, 1976.
20. Lefkowitz RJ, Mullikin D, Wood CL, Gore TB, Mukherjee C: J Biol Chem 252:5295, 1977.
21. Wessels MR, Mullikin D, Lefkowitz RJ: J Biol Chem (In press).
22. Lefkowitz RJ, Williams LT, Mullikin D: Molec Pharmacol (In press).
23. Schramm M, Rodbell MJ: Biol Chem 250:2232, 1975.
24. Levitzki A, Sevilla N, Steer ML: J Supramol Struct 4:405, 1976.