MOLECULAR PHARMACOLOGY, 16, 10-20

Selective Alteration in High Affinity Agonist Binding: A Mechanism of *Beta*-Adrenergic Receptor Desensitization

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(Received October 16, 1978)
(Accepted January 26, 1979)

SUMMARY

WESSELS, MICHAEL R., DEBRA MULLIKIN, AND ROBERT J. LEFKOWITZ: Selective Alteration in High Affinity Agonist Binding: A Mechanism of *Beta*-Adrenergic Receptor Desensitization. *Mol. Pharmacol.* **16**, 10-20, (1979).

Beta-adrenergic receptor desensitization of intact frog erythrocytes results in a greater percent fall in agonist ((±)[^{3}H]hydroxybenzylisoproterenol) than in antagonist ((−)[^{3}H]-dihydroalprenolol) binding. We now report the results of detailed studies on the alterations in agonist binding associated with *beta*-adrenergic receptor desensitization. Competition binding experiments revealed a lower overall affinity of agonist binding after desensitization. The affinity of antagonist ([^{3}H]dihydroalprenolol) binding appeared unchanged after desensitization. Three reagents (N-ethylmaleimide, EDTA, and Gpp(NH)p) that decrease high affinity agonist binding were shown to have less effect on [^{3}H]hydroxybenzylisoproterenol binding after desensitization. Two such agents (N-ethylmaleimide and dicyclohexyl carbodiimide) were also shown to block desensitization of intact cells. Guanine nucleotides failed to restore *beta*-adrenergic antagonist ([^{3}H]dihydroalprenolol) binding sites after desensitization. Adenylate cyclase catalytic activity was not impaired by desensitization and remained normally responsive to guanine nucleotides. These findings are consistent with a model of *beta*-adrenergic receptor desensitization in which desensitized receptors arise from receptors in the high affinity state, with chronic occupancy by agonist. The process of desensitization thus appears to result in selective loss or inactivation of these high affinity agonist binding sites.

INTRODUCTION

Decreased responsiveness to hormonal stimulation following chronic exposure to an agonist drug is a property common to many hormone sensitive cells (1-17). This phenomenon of "desensitization" has been observed in several systems possessing adenylate cyclase coupled *beta*-adrenergic receptors (1-14). Direct binding studies using specific *beta*-adrenergic antagonist ligands such as [^{3}H]DHA[2] (4-8) and [^{125}I]IHYP (12, 14) have shown that decreased responsiveness of adenylate cyclase after desensitization to *beta*-adrenergic stimulation is often accompanied by a fall in receptor number. *Beta*-adrenergic receptor desensitization has been studied in our laboratory using a frog erythrocyte model system, in which

This work was supported by Grants from DHEW #HL20339 and HL16037 and a grant-in-aid from the American Heart Association.

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[2] The abbreviations used are: [^{3}H]DHA, (−)[^{3}H]-dihydroalprenolol; [^{125}I]IHYP, (±)-[^{125}I]iodohydroxybenzylpindolol; [^{3}H]HBI, (±)-[^{3}H]hydroxybenzylisoproterenol; Gpp(NH)p, guanyl-5'-yl-imidodiphosphate; NEM, N-ethylmaleimide; DCCD, N,N' dicyclohexyl carbodiimide.

0026-895X/79/040010-11$02.00/0

incubation of the cells with isoproterenol for several hours results in a fall in [^{3}H]-DHA binding sites, as well as a decrease in isoproterenol stimulated adenylate cyclase activity (4, 7). In this report the term desensitization is used exclusively to mean the loss of binding capacity and hormone-stimulated adenylate cyclase activity brought about by chronic exposure of intact cells to agonist hormones. Incubation of cell lysates or membrane fractions with agonist hormones also leads to an apparent loss of binding capacity, but this latter phenomenon is due to the high affinity, slowly reversible binding of the agonist drug, which remains bound to a significant portion of the membrane receptors, even after washing (18). Residually bound agonist does not account for the loss of binding capacity induced by incubation of whole cells with agonist drugs (31).

Although it was appealing to postulate that the decrease in hormone sensitive adenylate cyclase activity was a direct result of the decreased number of *beta*-adrenergic receptors, we and others (4, 7, 8, 12, 14) have observed that the percent fall in catecholamine stimulated adenylate cyclase activity is consistently greater in magnitude than that in receptor number, as assayed by antagonist binding. Since catecholamine stimulation of adenylate cyclase activity is a function of *agonist*, and not antagonist binding, we have used the specific *beta*-adrenergic agonist radioligand [^{3}H]HBI, as well as the antagonist [^{3}H]DHA, to study receptor changes during *beta*-adrenergic receptor desensitization.

Direct binding studies using the specific *beta*-adrenergic agonist ligand [^{3}H]HBI have revealed several distinctive features of the interaction between an agonist drug and the *beta*-adrenergic receptor, not shared by antagonist-receptor interaction. In particular, (1) binding of [^{3}H]HBI to frog erythrocyte membranes is of high affinity, and is only slowly reversible, (2) a large portion of [^{3}H]HBI binding is rapidly dissociated in the presence of guanine nucleotides, and (3) interventions which perturb adenylate cyclase function or receptor-enzyme coupling also decrease [^{3}H]HBI binding. Observation of these features of agonist binding has led to the development of a model of hormone-receptor interaction (18) in which agonist binds in a reversible fashion to the *beta*-adrenergic receptor; this relatively low affinity form of the receptor is converted to a high affinity state from which the agonist dissociates only very slowly. The high affinity form of the receptor is associated with receptor-enzyme coupling, and, in the presence of guanine nucleotides, the high affinity receptor-agonist complex is rapidly dissociated with concomitant activation of the coupled adenylate cyclase. Under our usual assay conditions (1–100 nM [^{3}H]HBI, no nucleotide) the agonist appears to label predominantly the high affinity form of the receptors, resulting in specific [^{3}H]HBI binding to about 60% of the total receptor sites labeled by the antagonist [^{3}H]DHA (8).

Our recent finding that *beta*-adrenergic receptor desensitization appears to result in the functional loss of an equal absolute number of agonist and antagonist sites, but a larger percentage of high affinity agonist sites (8) led us to speculate that the receptor sites lost during desensitization might arise exclusively from that population of receptors exhibiting high affinity agonist binding. We now report the results of detailed studies designed to examine more closely the alterations in agonist binding associated with *beta*-adrenergic receptor desensitization. The findings support the hypothesis that *beta*-adrenergic receptor desensitization involves selective loss or inactivation of high affinity agonist binding sites.

METHODS

Blood from southern grass frogs was collected and washed as previously described (4). Pooled erythrocytes were incubated at 23° with or without 0.1 mM (−)isoproterenol (+)bitartrate in a medium containing 101 mM NaCl, 17 mM Tris-HCl, 10 mM glucose, and 0.2 mM sodium metabisulfite at pH 7.4 for varying periods of time. Incubations were shielded from light, and cells kept in suspension by slow rotation. Following incubation, cells were washed three times in buffered "amphibian saline" (100 mM NaCl, 10 mM Tris-HCl, pH 7.4) at 4°, and membrane fractions were prepared.

Two membrane preparations were used

in these experiments: a "crude" preparation, consisting of a washed lysate of the cells (8), and a "purified" membrane fraction, prepared by centrifugation of cell lysates over 50% sucrose (18).

Assays for [^{3}H]HBI binding (19), [^{3}H]-DHA binding (20), and adenylate cyclase (21) were performed as previously described, with modifications as indicated in figure legends. All assays contained 50 mM Tris-HCl, pH 7.4, and 16 mM $MgCl_2$. Protein concentration was generally 2–4 mg/ml in binding assays, and 1–2 mg/ml in adenylate cyclase assays. All binding data are reported as "specific binding," defined as total radioligand bound minus radioligand bound in the presence of 10 μM propranolol, except where otherwise indicated in figure legends.

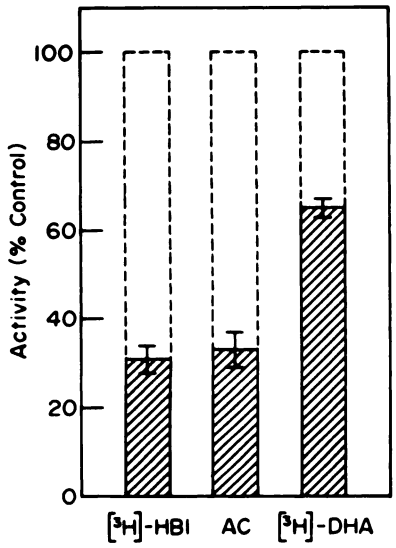

FIG. 1. *Isoproterenol-induced desensitization of beta-adrenergic receptors and catecholamine-sensitive adenylate cyclase*

Crude membrane fractions prepared from control and desensitized (5 hr) cells were assayed for [^{3}H]HBI binding, [^{3}H]DHA binding, and isoproterenol stimulated adenylate cyclase activity. [^{3}H]HBI was present at 6 nM and [^{3}H]DHA at 10 nM in binding assays. Control values were 46 and 126 fmol bound/mg protein, respectively. Isoproterenol-stimulated adenylate cyclase activity was defined as the activity of the enzyme stimulated by 0.1 mM isoproterenol above basal activity, and was 623 pmol of cAMP/min/mg in controls, representing a 10.2 fold stimulation of basal activity. Values shown represent the mean ± S.E. of triplicate determinations in three separate experiments.

RESULTS

Agonist vs antagonist binding after desensitization. Figure 1 summarizes the basic observation (8) that stimulated the present studies. After desensitization of frog erythrocytes by a several-hour exposure to isoproterenol, there is, on a percentage basis, a greater fall in agonist ([^{3}H]HBI) than antagonist ([^{3}H]DHA) binding. The percent fall in agonist binding approximates that in catecholamine-stimulated adenylate cyclase.

Affinity of agonist binding. The overall affinity of agonist binding to the *beta*-adrenergic receptors was examined by competition experiments using an unlabeled agonist (isoproterenol) and labeled antagonist [^{3}H]DHA. This approach permits an examination of the full range of agonist concentrations as opposed to the more limited range of concentrations which can be used with direct agonist binding studies. In such competition curves of isoproterenol competing with a constant concentration of [^{3}H]DHA for binding sites (Fig. 2A) a two-fold higher concentration of isoproterenol was required to compete for 50% of [^{3}H]-DHA binding following desensitization (IC_{50}, control = 1.2 μM; IC_{50}, desensitized = 2.3 μM), suggesting the overall affinity of agonist binding may be lower after desensitization. In the presence of 30 μM Gpp(NH)p, a nonhydrolyzable GTP analogue, the control and desensitized competition curves were both shifted to the right, and showed essentially identical affinities (IC_{50} = 6.5 μM).

In contrast, similar competition curves for the antagonist propranolol competing for binding sites with [^{3}H]DHA showed identical curves for control and desensitized preparations (IC_{50} = 35 nM, Fig. 2B). The affinity of antagonist binding was not altered by the presence of guanine nucleotides (data not shown).

[^{3}H]DHA binding after preincubation of membranes with isoproterenol. In previous studies (22) we have demonstrated that incubation of frog erythrocyte membranes with isoproterenol followed by washing leads to a fall in the number of [^{3}H]DHA binding sites which can be assayed in the

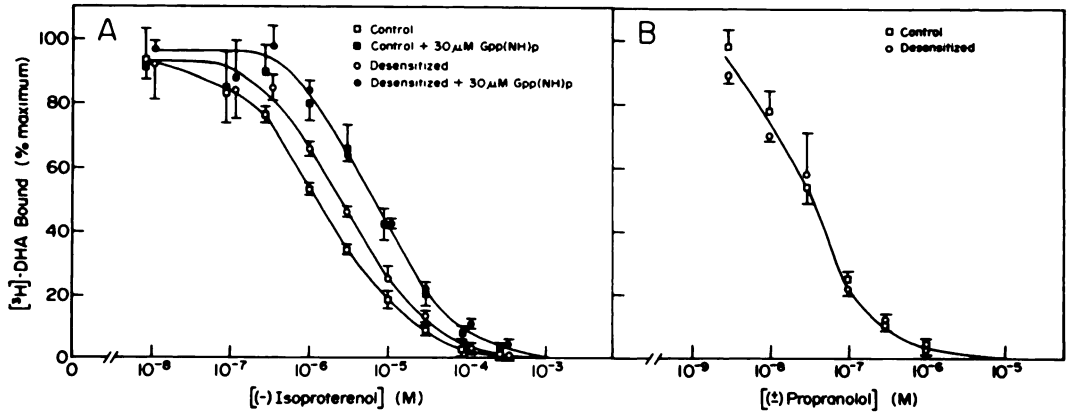

FIG. 2. *Agonist and antagonist competition for [³H]DHA binding sites*

Crude membrane fractions were prepared from desensitized (3–5 hr) and control cells. The membrane fractions were assayed for [³H]DHA binding in the presence of the indicated concentrations of isoproterenol, with or without 30 μM Gpp(NH)p (Panel A), or in the presence of the indicated concentrations of propranolol (Panel B). [³H]DHA was present at 5 nM in all assays. Data points represent mean values ± S.E. from triplicate determinations in two separate experiments for each panel, expressed as percent maximum binding for each condition. Maximum binding was defined as total [³H]DHA bound in the absence of competing ligand minus [³H]DHA bound in the presence of 1 mM isoproterenol (Panel A) or 10 μM propranolol (Panel B). Maximum [³H]DHA binding amounted to 120–250 fmol/mg protein in controls, 20–40% less in desensitized preparations.

membranes. We have shown (18) that this reduction is a consequence of high affinity, slowly dissociable binding of the agonist to the receptors.

When membrane fractions prepared from control cells were incubated with isoproterenol a 42% reduction in the apparent number of [³H]DHA binding sites resulted, presumably due to tightly bound isoproterenol occupying 42% of the receptors (Fig. 3). However, isoproterenol preincubation of membranes from desensitized cells resulted in only a 17% reduction in [³H]DHA sites (Fig. 3). In other words, a smaller fraction of the total receptor population remaining after desensitization is capable of binding agonist with high affinity.

Effects of reagents that interfere with agonist binding. [³H]HBI binding following desensitization was found to be relatively resistant to the effects of three interventions which have been shown to decrease the overall affinity of agonist binding. When membrane fractions from desensitized cells were treated with 5 or 10 mM NEM, a sulfhydryl reagent which interferes with adenylate cyclase function (18), a smaller percentage of [³H]HBI binding sites were lost than in control preparations (Fig. 4A). Similarly, when Mg^{2+} was depleted from the binding assay mixture with increasing concentrations of EDTA (23), [³H]HBI binding to membrane fractions from desensitized cells was relatively less affected than binding in controls (Fig. 4B). Increasing concentrations of Gpp(NH)p (18) also had relatively less effect in diminishing [³H]HBI binding following desensitization (Fig. 4C). In general, with each of these reagents, the difference in [³H]HBI binding between control and desensitized preparations became progressively smaller with increasing concentrations of the reagent. In the presence of saturating concentrations of Gpp(NH)p, there appears to be no difference between the level of [³H]HBI binding to membranes from control and desensitized cells.

In all of the experiments depicted in Fig. 4 [³H]DHA binding was also measured. NEM, EDTA and Gpp(NH)p at the concentrations tested did not affect [³H]DHA binding to either control or desensitized preparations (data not shown).

Ability of agents that interfere with agonist binding to block desensitization. Pretreatment of cells with 10 mM NEM effectively prevented the loss of [³H]DHA binding sites normally induced by subsequent incubation of the cells with isoproterenol

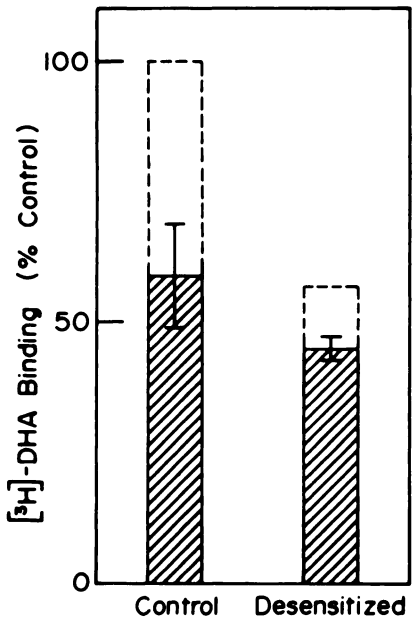

FIG. 3. *[^{3}H]DHA binding after preincubation of membranes with isoproterenol*

Crude membrane fractions from desensitized (5 hr) and control cells were incubated at 25° for 30 min with or without 0.1 mM isoproterenol. The membranes were then washed twice to remove free isoproterenol, and assayed for [^{3}H]DHA binding. The open bars indicate the number of [^{3}H]DHA binding sites in membrane fractions not incubated with isoproterenol; the hatched bars show the number of [^{3}H]DHA binding sites remaining after incubation of the membranes with isoproterenol. Binding is expressed as percent of specific binding in control membranes incubated without isoproterenol. Data shown represent mean value ± S.E. from duplicate determinations in three separate experiments. Number of [^{3}H]DHA binding sites was determined by Scatchard analysis of specific binding for each membrane preparation and amounted to 100–250 fmol/mg of protein in control preparations.

(Fig. 5). Treatment of cells with this concentration of NEM results in greater than 90% loss of adenylate cyclase activity and a marked reduction in [^{3}H]HBI binding (data not shown).

Pretreatment of frog erythrocytes with the lipid soluble reagent dicyclohexyl carbodiimide (DCCD) has been shown to abolish catecholamine stimulated adenylate cyclase, while PGE_1, fluoride and basal adenylate cyclase remain unaffected (24). Concentrations (10 μM) of the reagent that abolish catecholamine stimulated adenylate cyclase markedly lower [^{3}H]HBI binding without affecting [^{3}H]DHA binding (24). As shown in Fig. 6, pretreatment of cells with 10 μM DCCD prevents isoproterenol-induced reduction in [^{3}H]DHA binding. In these experiments control cells pretreated with NEM or DCCD showed a small (5–10%) decrease in [^{3}H]DHA binding from controls not treated with NEM or DCCD; in contrast, treated cells subsequently incubated with isoproterenol usually showed a small (0–15%) increase in [^{3}H]DHA binding over untreated controls, and a larger increase over NEM or DCCD treated controls, suggesting that isoproterenol may have a stabilizing effect on the receptor in its "uncoupled" state.

Guanine nucleotide effects on [^{3}H]DHA binding. Performing binding assays in the presence of 1.0 mM GTP or 0.1 mM Gpp(NH)p had no effect on the apparent number of [^{3}H]DHA binding sites in lysates of desensitized cells (Table 1). The degree of desensitization observed was identical under all assay conditions (p = NS, paired t-test, n = 3 experiments). These experiments serve to distinguish the desensitized state of the *beta*-receptor formed in intact cells from the high affinity state of the receptor formed in isolated membranes upon exposure to *beta*-agonists. The latter form of the receptor, which also cannot be labeled by [^{3}H]DHA (due to tight agonist occupancy), is "regenerated" by guanine nucleotides, which lower agonist affinity and lead to rapid dissociation of the agonist from the receptors (18, 25).

Guanine nucleotide effects on adenylate cyclase. The presence of 0.1 mM GTP augmented the rates of isoproterenol stimulated cAMP production in control and desensitized preparations to approximately the same degree (Fig. 7); thus, GTP showed no tendency to restore catecholamine-stimulated cyclase in the desensitized preparation back to control levels.

In the presence of 10 μM Gpp(NH)p, isoproterenol-stimulated adenylate cyclase showed identical rates of cAMP production in lysates of control and desensitized cells (Fig. 8). However, in the absence of isoproterenol the rate of cAMP production in

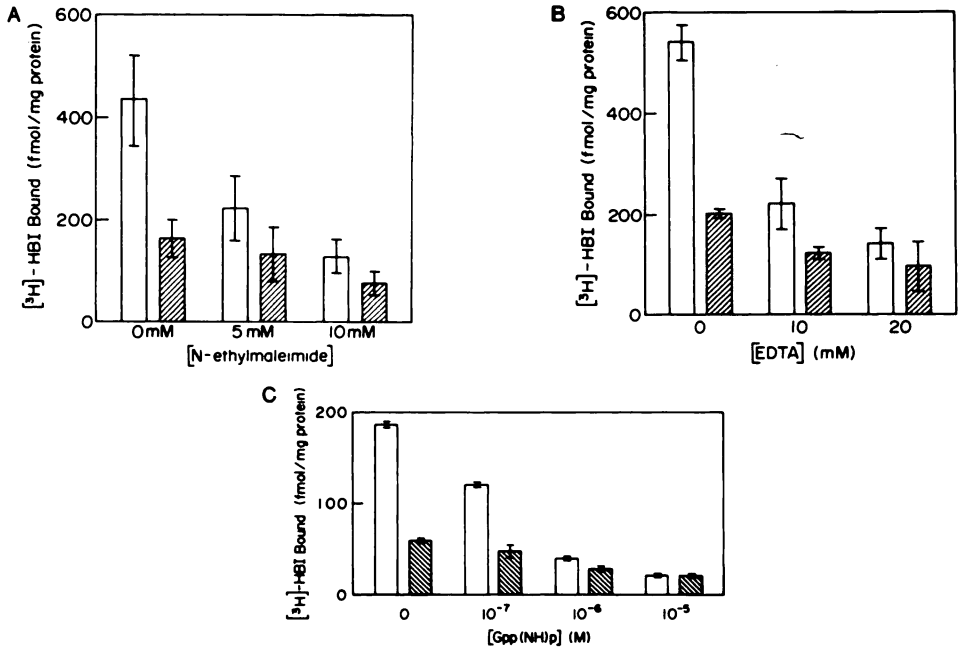

FIG. 4. *Effect of NEM, EDTA, and Gpp(NH)p on [^{3}H]HBI binding*

Panel A) Effect of NEM. Purified membrane fractions were prepared from desensitized (3 hr) and control cells. The membrane fractions were then incubated for 10 min at 25° in the presence of the indicated concentrations of NEM, then assayed for [^{3}H]HBI binding in the presence of 28 nM [^{3}H]HBI. Data shown represent mean values ± S.E. of duplicate determinations in two separate experiments for control (open bars) and desensitized (hatched bars) preparations. Panel B) Effect of EDTA on [^{3}H]HBI binding. Purified membrane fractions were prepared from desensitized (3 hr) and control cells. The membrane fractions were then assayed for [^{3}H]HBI binding in the presence of 3 mM Mg and the indicated concentrations of EDTA. [^{3}H]HBI was present at 28 nM. Data shown represent mean values ± S.E. of duplicate determinations in two separate experiments for control (open bars) and desensitized (hatched bars) preparations. Panel C) Effect of Gpp(NH)p on [^{3}H]HBI binding. Purified membrane fractions were prepared from desensitized (5 hr) and control cells. The membrane fraction was then assayed for [^{3}H]HBI binding in the presence of the indicated concentrations of Gpp(NH)p. [^{3}H]HBI was present at 6 nM. Data shown represent the range of mean values of duplicate determinations in two separate experiments, for control (open bars) and desensitized (hatched bars) preparations.

both control and desensitized preparations was the same and progressively approached that of hormone stimulated activity, eventually attaining the same rate of enzyme activity (Fig. 8).

DISCUSSION

We and others have shown that competition curves for unlabeled agonist competing for *beta*-adrenergic binding sites with a labeled antagonist are shallower than antagonist competition curves. In the presence of guanine nucleotides the agonist competition curve is shifted to the right, and becomes steeper, assuming the shape of an antagonist curve (26, 27). The experiments reported here demonstrate that the agonist competition curve following desensitization is shifted to the right, and in the presence of Gpp(NH)p, the curve is further shifted to the same position as the control curve in the presence of nucleotide (Fig. 2). These results are explicable in terms of the model outlined above: under the conditions of these experiments it appears that only about 60% of the total population of *beta*-adrenergic receptors bind agonist with high affinity, while the remaining receptors bind agonist with relatively low affinity. So, the agonist competition curve represents a hybrid of two affinities of agonist binding. Desensitization involves selective loss of

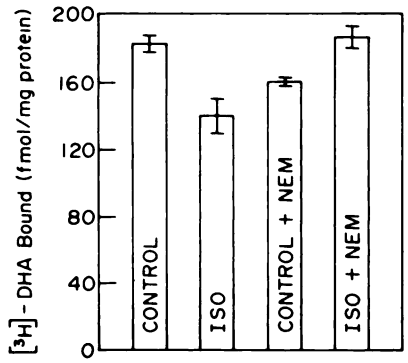

FIG. 5. *Inhibition of desensitization by pretreatment of cells with NEM*

Intact cells were incubated with or without 10 mM NEM for 15 min at 37°, washed twice, and "desensitized" for 2½ hours, as described under METHODS. Purified membrane fractions were prepared and assayed for [^{3}H]DHA binding in the presence of 40 nM [^{3}H]DHA. Data shown represent mean values ± S.E. of duplicate determinations in two separate experiments. The abbreviations used are Iso, isoproterenol; NEM, N-ethylmaleimide.

high affinity agonist binding sites, resulting in a greater relative contribution of the low affinity sites to the overall observed affinity of the desensitized curve. Since high affinity sites make up a smaller fraction of the total receptors to which agonist can bind after desensitization, the curve is shifted to the right. In the presence of Gpp(NH)p virtually all high affinity agonist binding is rapidly dissociated, so both control and desensitized curves show equal, lower affinity.

That desensitization involves selective depletion of high affinity agonist binding sites was also confirmed by a series of experiments designed to examine the high affinity component of agonist binding in membrane fractions of control and desensitized cells. Preincubation of membrane fractions with isoproterenol results in a decrease in the apparent number of [^{3}H]DHA binding sites, presumably because of tightly bound agonist occupying a portion of the receptors. The fraction of receptors which bind agonist with high affinity may be estimated from this apparent loss of [^{3}H]-DHA binding sites. Following desensitization, these high affinity agonist sites make up a much smaller fraction of the total population of receptors, 17% vs 42% in controls, in these experiments (Fig. 3).

If the total amount of agonist binding after desensitization has a relatively smaller component of high affinity binding, the overall level of agonist binding after desensitization should be less affected by perturbations which affect high affinity binding. This prediction was confirmed by the results of experiments in which membrane fractions from desensitized cells were assayed for [^{3}H]HBI binding in the presence of each of three reagents known to decrease high affinity agonist binding: (1) NEM, a sulfhydryl reagent which decreases adenylate cyclase activity and [^{3}H]HBI binding (18), (2) EDTA, which depletes Mg^{2+}, a cation required for high affinity agonist binding (23), and (3) Gpp(NH)p, a guanine nucleotide which causes a rapid dissociation of a large portion of high affinity agonist binding (18). Although each reagent had some effect in reducing [^{3}H]HBI binding in membrane fractions from desensitized cells, in each case the effect was less pronounced than in controls (Fig. 4).

The level of [^{3}H]HBI binding in control and desensitized preparations tended to become more nearly equal at higher concentrations of each reagent (Fig. 4); this effect is consistent with a progressive elimination

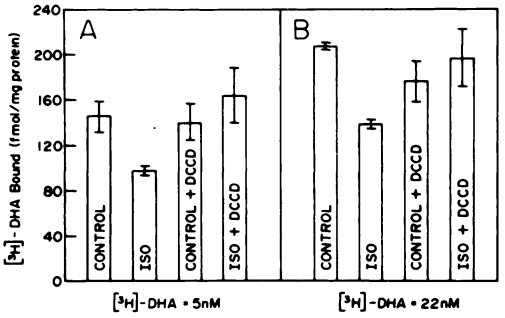

FIG. 6. *Inhibition of desensitization by pretreatment of cells with DCCD*

Intact cells were preincubated with or without 10 μM DCCD for 15 min at 25°, then "desensitized" with 0.1 mM isoproterenol for 3 hr, as described under METHODS. Crude membrane fractions were prepared and assayed for [^{3}H]DHA binding in the presence of the indicated concentrations of [^{3}H]DHA. Data shown represent mean values ± S.E. of duplicate determinations in two separate experiments. The abbreviations used are Iso, isoproterenol; DCCD, N,N′ dicyclohexyl carbodiimide.

TABLE 1

Effect of guanine nucleotides on [^{3}H]DHA binding

Purified (experiment A) or crude (experiments B and C) membrane fractions were prepared from desensitized (5 hours) and control cells. Membranes were assayed for [^{3}H]DHA binding in the presence or absence of 0.1 mM Gpp(NH)p or 1.0 mM GTP. [^{3}H]DHA was present at 8–10 nM. Data shown represent mean values of duplicate determinations in each experiment. Figures in parentheses represent binding expressed as percent of corresponding control.

	[^{3}H]dihydroalprenolol binding					
	No addition		1.0 mM GTP		0.1 mM Gpp(NH)p	
	Control	Desensitized	Control	Desensitized	Control	Desensitized
	(fmol/mg protein)					
Experiment A	298	216 (72%)	318	190 (60%)	298	225 (76%)
Experiment B	67.4	48.2 (72%)	61.9	44.2 (71%)	63.7	50.2 (79%)
Experiment C	185	120 (65%)	178	131 (74%)	184	138 (75%)
Mean		70%		68%		77%

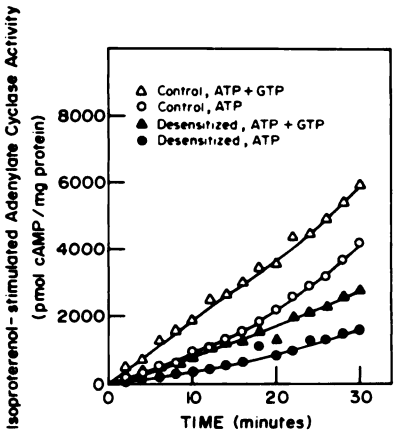

FIG. 7. *Effect of GTP on isoproterenol-stimulated adenylate cyclase activity*

Crude membrane fractions prepared from desensitized (5 hr) and control cells were incubated at 37° with 0.1 mM isoproterenol in the presence of 1.0 mM ATP alone or in combination with 1.0 mM GTP, in a standard adenylate cyclase assay mixture, as described under METHODS. Aliquots were withdrawn at the indicated time points and cAMP production measured. Isoproterenol-stimulated adenylate cyclase activity was defined as the increase in cAMP production above basal activity. Data points from one experiment are shown; similar results were obtained in a second experiment.

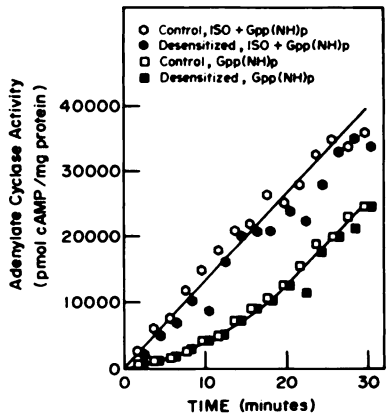

FIG. 8. *Activation of adenylate cyclase by Gpp(NH)p*

Crude membrane fractions prepared from desensitized (5 hr) and control cells were incubated at 37° in the presence of 0.1 mM Gpp(NH)p and 1.0 mM ATP, with or without 0.1 mM isoproterenol, in a standard adenylate cyclase assay mixture, as described under METHODS. Aliquots were withdrawn at the indicated time points and cAMP production measured. Data points from one experiment are shown; similar results were obtained in a second experiment.

of the contribution of high affinity agonist sites to the total observed binding. The difference in total [^{3}H]HBI binding between control and desensitized preparations is presumed to be due to the difference in number of high affinity binding sites. As these sites are functionally removed by treatment with the agents discussed above, the difference between control and desensitized levels of agonist binding is removed, as well. Thus, the results of these experiments tend to confirm the conclusions drawn from agonist competition curves.

Our model of desensitization also predicts that perturbations of intact cells that interfere with high affinity agonist binding will

block desensitization, since desensitized receptors are thought to arise exclusively from the high affinity state of the receptor with chronic occupancy by agonist. Two such treatments of intact cells, incubation with NEM or DCCD, effectively blocked the loss of [^{3}H]DHA binding sites normally induced by chronic exposure to isoproterenol (Fig. 5, 6).

The effects of guanine nucleotides on adenylate cyclase activity following desensitization suggest that the diminished enzyme responsiveness to hormonal stimulation is a result of receptor alteration, rather than any intrinsic defect in the enzyme. GTP augmented hormone-stimulated activity in lysates of desensitized cells, but the relationship of desensitized to control activity remained relatively constant, as control activity was potentiated to a similar extent (Fig. 7). In contrast, Gpp(NH)p might appear to "resensitize" hormone stimulated adenylate cyclase activity, as the desensitized preparation showed the same rate of hormone stimulated activity as control in the presence of Gpp(NH)p (Fig. 8). However, since the basal rate of cyclase activity in the presence of Gpp(NH)p eventually attains the same rate as that observed in the presence of isoproterenol, it appears that the apparent "resensitization" is largely independent of receptor-enzyme interaction. Agonist stimulation via *beta*-adrenergic receptors presumably facilitates the activation of adenylate cyclase by Gpp(NH)p, but even in the absence of hormone stimulation, maximal enzyme activation is eventually achieved (Fig. 8). Apparently, even the diminished number of functional receptors remaining after desensitization is sufficient to produce virtually immediate attainment of the maximal Gpp(NH)p stimulated rate of adenylate cyclase activity. This finding is analogous to the results of Schramm who reported the ability of Gpp(NH)p to restore catecholamine stimulated adenylate cyclase activity in turkey erythrocyte membranes in which 97% of hormone-stimulated adenylate cyclase activity had been lost by pre-treatment with DCCD (28). That guanine nucleotides do not in any true sense "resensitize" *beta*-adrenergic receptors is further confirmed by the finding that neither GTP nor Gpp(NH)p has any effect on the apparent number of [^{3}H]DHA binding sites in lysates of desensitized cells (Table 1).

We have fit our current conceptualization of the mechanism of desensitization into a general model of agonist-receptor interaction in the following way (Fig. 9): agonist (Ag) binding to the receptor (R) promotes association of the receptor with some component(s) of adenylate cyclase (AC) and results in high affinity, slowly reversible binding of the agonist. In the presence of guanine nucleotides (nuc.) the high affinity complex is rapidly dissociated, concomitant with activation (*) of adenylate cyclase. Rapid cycling through the binding-activation-dissociation sequence presumably accounts for the high efficiency receptor-cyclase coupling observed in membrane preparations stimulated by agonist drugs in the presence of guanine nucleotides (29, 30). Desensitized receptors (R_D) arise exclusively from the high affinity state of the receptor with chronic occupancy by agonist. In its desensitized form the receptor

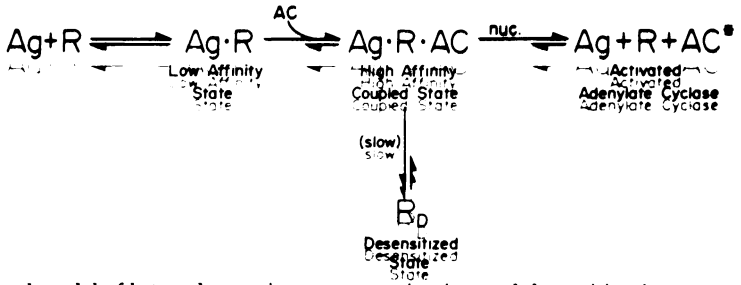

FIG. 9. *Proposed model of beta-adrenergic receptor activation and desensitization*
Abbreviations used are Ag, agonist; R, *beta*-adrenergic receptor; AC, some component(s) of adenylate cyclase; AC*, activated adenylate cyclase; nuc., guanine nucleotide; R_D, desensitized beta-adrenergic receptor.

binds neither agonist nor antagonist and cannot stimulate adenylate cyclase. It has been shown previously that this loss of binding capacity is not accounted for by residually bound desensitizing drug (31).

The model outlined above seems to us the most parsimonious explanation of the findings presented here; however, other hypothetical mechanisms are equally consistent with our data. For example, desensitization might involve a lesion which impairs receptor-cyclase coupling, or otherwise prevents formation of the high affinity agonist-receptor complex, resulting in a fall in agonist binding with little or no effect on antagonist binding. An alteration of this type which interferes with formation of the high affinity agonist-receptor complex would be expected to result in a decrease in the overall affinity of agonist binding (cf. Fig. 2), because fewer receptors would bind agonist with high affinity. In the frog erythrocyte system the hormone binding site itself also must be affected to some degree, either by the same lesion or a superimposed defect, since antagonist binding is also decreased by desensitization.

Certain fundamental questions remain unanswered with regard to the mechanisms of *beta*-adrenergic receptor activation and desensitization. For example, we do not know yet which molecular components of the receptor-cyclase system are necessary for each step of the binding-activation-desensitization sequence; nor do we know what molecular alterations in the receptors render them desensitized. Another area of uncertainty relates to the observation that only a portion of the total population of *beta*-adrenergic receptors bind agonist with high affinity. Several explanations seem plausible. Fewer cyclase than receptor moieties or membrane constraints on receptor-cyclase coupling might limit the number of high affinity complexes formed at one time; or, steric interactions between receptors may be induced by high affinity agonist binding, interfering with subsequent binding to unoccupied receptors. Residual guanine nucleotides present in the membrane preparations may lead to rapid dissociation of agonist from a portion of the receptors. Since guanine nucleotides are present in the intact cell, the high affinity state of the receptor must be very short-lived under physiologic conditions. Perhaps under these conditions all *beta*-adrenergic receptors may freely cycle through the high affinity state in the presence of agonist.

Because of the relatively low affinity of [^{3}H]HBI binding in the presence of guanine nucleotides, it is technically difficult to perform binding assays under conditions in which agonist binding results in adenylate cyclase stimulation. However, this limitation in our methods is counterbalanced by the fact that binding studies performed in the absence of guanine nucleotides have allowed us to identify the high affinity state of the *beta*-adrenergic receptor and to characterize its apparently central role in *beta*-adrenergic receptor desensitization.

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