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Adenylate Cyclase-Coupled *Beta* Adrenergic Receptors: Effect of Membrane Lipid-Perturbing Agents on Receptor Binding and Enzyme Stimulation by Catecholamines

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SUMMARY

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Binding of (−)-[^{3}H]dihydroalprenolol, a potent competitive *beta* adrenergic antagonist, to sites in frog erythrocyte membranes has previously been demonstrated to possess the essential properties expected of binding to adenylate cyclase-coupled *beta* adrenergic receptors. The present studies were designed to test the effects of a variety of membrane lipid-perturbing agents on both *beta* adrenergic receptor binding and catecholamine-responsive adenylate cyclase in frog erythrocyte membranes. Digestion of membranes with phospholipases A, C, and D causes a dose-dependent decline in receptor binding capacity without altering receptor affinity. Amphotericin B, a nondegradative membrane lipid perturbant, also causes a dose-dependent decrease in (−)-[^{3}H]dihydroalprenolol binding. Decrements in catecholamine-stimulated adenylate cyclase activity caused by these agents are always greater than decreases in basal and fluoride-sensitive enzyme activities. Decreases in (−)-[^{3}H]dihydroalprenolol binding parallel the disproportionate reduction in catecholamine responsiveness of adenylate cyclase. By contrast, the polyene antibiotic Filipin appears to "uncouple" receptor binding and enzyme activation, since a marked reduction in isoproterenol-stimulated adenylate cyclase is not accompanied by a decrease in specific (−)-[^{3}H]dihydroalprenolol binding.

INTRODUCTION

Numerous studies have documented the feasibility of identifying membrane-bound macromolecular hormone and drug receptors by using radiolabeled hormones, drugs, and analogues. Recent reports from this and other laboratories have described methods for labeling *beta* adrenergic receptors in membrane fractions with radiolabeled *beta* adrenergic antagonists (1-5). We have used (−)-[^{3}H]dihydroalprenolol, a potent competitive *beta* adrenergic antagonist, for this purpose. The binding of this radiolabeled ligand to sites in membranes of frog erythrocytes (1, 2), canine heart (6), rat adipocytes (7), and human lymphocytes (8) has the characteristics expected of binding to the *beta* adrenergic receptors which are linked to adenylate cyclase in these membranes. The binding is rapid, saturable, readily reversible, and exhibits strict stereospecificity for the (−) enantiomers of *beta* adrenergic agonists and antagonists. In addition, the potency

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series of agonists and antagonists for inhibition of binding and interaction with adenylate cyclase are identical. Thus the (−)-[^{3}H]dihydroalprenolol binding sites appear to be equivalent to the adenylate cyclase-coupled *beta* adrenergic receptor binding sites in frog erythrocyte membranes.

The experiments reported here were designed to test the effects of a variety of lipid-perturbing agents on both *beta* adrenergic receptor binding [i.e., specific (−)-[^{3}H]dihydroalprenolol binding] and catecholamine-responsive adenylate cyclase in purified frog erythrocyte membranes. The ability to study in parallel both *beta* adrenergic receptor binding and catecholamine-stimulated adenylate cyclase activity permits a more direct assessment of the loci of lipid-perturbing effects on this catecholamine-responsive system than has previously been possible

METHODS

Materials. (−)-Isoproterenol, (±)-propranolol, cAMP,[2] ATP, phosphoenolpyruvate, phospholipase A (bee venom, 1180 units/mg; *Vipera russelli*, 6 units/mg), phospholipase D (cabbage, 22 units/mg), phospholipase C (*Clostridium welchii*, 5 units/mg), myokinase, and nystatin were obtained from Sigma Chemical Company; pyruvate kinase, from Calbiochem; and amphotericin B, from Squibb. Filipin was a generous gift of G. B. Whitfield of the Upjohn Company.

[^{3}H]cAMP (1–5 Ci/mmole) and [α-^{32}P]ATP (1–10 Ci/mmole) were obtained from New England Nuclear Corporation. (−)-Alprenolol hydrochloride was obtained from Hassle Pharmaceuticals and tritiated by New England Nuclear by catalytic reduction to a specific radioactivity of 17–33 Ci/mmole. (−)-Alprenolol has an unsaturated bond between the second and third carbon atoms in the aliphatic chain on position 2 of the aromatic ring. The structure of the tritiated product is that of (−)-[^{3}H]dihydroalprenolol. This structure was verified by mass spectroscopy (9). (−)-[^{3}H]Dihdroalprenolol has biological activity identical with that of native (−)-alprenolol (9).

Grass frogs were obtained from Carolina Biological Company or Nasco-Steinhilber.

Alumina, neutral grade, was obtained from Nutritional Biochemicals. Dowex AG 50W-X4 was a product of Bio-Rad.

Membrane preparations. Blood from grass frogs maintained at 23° was collected from the aorta while the frog was infused with heparinized NaCl through the ventral vein. The cells were washed three times with a solution of 110 mM NaCl–10 mM Tris-HCl, pH 7.4, by centrifugation at 200 × *g* in the cold. Cells were lysed in 1 mM dithiothreitol and then placed in 7.5 mM Tris-HCl–2.5 mM MgCl, pH 8.1, prior to centrifugation at 30,000 × *g* for 15 min at 4°. Membranes were resuspended in 10 mM Tris-HCl–10 mM $MgCl_2$–1 mM dithiothreitol, pH 8.1, by homogenization, and centrifuged over 50% sucrose in 10 mM Tris-HCl–10 mM MgCl–1 mM dithiothreitol, pH 8.1, for 15 min at 800 × *g* and 4°. The supernatant was collected and centrifuged at 30,000 × *g* for 15 min at 4°. The pellet was washed once by homogenization and recentrifugation in 10 mM Tris-HCl–10 mM $MgCl_2$–1 mM dithiothreitol, pH 8.1, and then resuspended in the appropriate incubation buffer, 75 mM Tris-HCl–25 mM $MgCl_2$, pH 8.1, unless otherwise noted.

Membranes were prepared fresh for each experiment.

(−)-[^{3}H]Dihydroalprenolol binding assay. The binding assay was performed as described previously (1, 2), with minor modifications. Membrane suspensions, 100 µl, usually containing 450–700 µg of protein, were incubated with 50,000–80,000 cpm of (−)-[^{3}H]dihydroalprenolol (13–21 nM, final concentration) in a final volume of 250 µl containing 25 mM Tris-HCl–10 mM $MgCl_2$, pH 8.1. At the end of the 10-min, 37° incubation, duplicate 50-µl aliquots were layered over 300 µl of the incubation buffer in small polyethylene tubes and centrifuged for 2 min in a Beckman Microfuge 152. Membranes pelleted almost immediately. After centrifugation, the supernatants were aspirated with the aid of a No. 20 spinal needle attached to a vacuum line, and the pellets were rinsed

[2] The abbreviations used are: cAMP, adenosine cyclic 3′,5′-monophosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N′*-tetraacetic acid.

with 500 μl of buffer. After the fluid was aspirated, the tips of the centrifuge tubes were cut off into scintillation vials, and the membrane pellets were dissolved by swirling overnight in 0.5 ml of 10% sodium dodecyl sulfate–10 mM EDTA. Then 10 ml of Triton-toluene-based scintillation fluid were added, and "bound" (−)-[^{3}H]dihydroalprenolol was determined by counting in a Packard liquid scintillation spectrometer.

In every experiment (−)-[^{3}H]dihydroalprenolol "nonspecifically" bound and/or trapped in the membrane pellets was determined by measuring the amount of radioactivity in pellets obtained when incubation was performed in the presence of 10 or 100 μM (±)-propranolol. The counts "nonspecifically" bound were subtracted from the total radioactivity bound to determine (−)-[^{3}H]dihydroalprenolol "specifically" bound, as described previously (1, 2). "Nonspecific" binding was 10–20% of the total radioactivity associated with the pellets when (−)-[^{3}H]dihydroalprenolol was present at 10–30 nM. In all figures and tables, bound (−)-[^{3}H]dihydroalprenolol refers to "specific" binding as defined above.

Preparation of frog erythrocyte membrane–polyene antibiotic suspensions. The polyene antibiotics Filipin, amphotericin B, and nystatin were dissolved in methanol, and appropriate quantities were dried as a thin coat on the bottom of 5-ml, round-bottomed flasks by rotation on ice under a stream of nitrogen. Purified frog erythrocyte membrane preparations (0.5 ml) were added to the polyene-coated vessel. The polyene was suspended in the aqueous membrane medium by swirling the flasks in a New Brunswick rotary shaker water bath at 25° for 10 min at 300 rpm (10).

Adenylate cyclase assay. Incubations were performed as previously described (2) in a volume of 50 μl which contained 25 mM Tris-HCl buffer (pH 8.1), 10 mM $MgCl_2$, 0.1 mM cAMP, 1.5 mM ATP, [α-^{32}P]ATP (1×10^6 cpm), 5 mM phosphoenolpyruvate, 10 μg/ml of myokinase, and 40 μg/ml of pyruvate kinase. Incubations were performed for 10 min at 37° and were stopped by the addition of 1 ml of a solution containing [^{3}H]cAMP (15,000 cpm/ml), 100 μg of ATP, and 50 μg of cAMP.

Final membrane protein concentration in the assay was 1.2–3.5 mg/ml. The adenylate cyclase assay was linear with time and with protein concentration over the range studied.

The [^{32}P]cAMP product formed was isolated by sequential chromatography on columns of Dowex AG50W-X4 and alumina as described by Salomon *et al.* (11). Samples were added to 10 ml of Triton-toluene-based scintillation fluid and counted in a Packard liquid scintillation spectrometer. Results were corrected for product recovery on the basis of [^{3}H]cAMP recovered.

(−)-[^{3}H]Dihydroalprenolol binding assays and adenylate cyclase assays were performed under comparable conditions except for the presence of ATP, cAMP, and an ATP-regenerating system in the adenylate cyclase assays. We have demonstrated (data not shown) that ATP, cAMP, and the ATP-regenerating system are without effect on steady-state binding. Protein was determined by the method of Lowry *et al.* (12).

RESULTS

Effects of phospholipase digestion on (−)-[^{3}H]dihydroalprenolol binding and isoproterenol-stimulated adenylate cyclase activity. Treatment of membranes with phospholipases consistently decreased specific binding of (−)-[^{3}H]dihydroalprenolol in a dose-dependent fashion (Fig. 1). The decline in "specific" binding resulted from both a decrease in total (−)-[^{3}H]dihydroalprenolol binding and an increase in nonspecific binding of radioligand to the membranes. Although Fig. 1 demonstrates effects elicited by phospholipase A derived from *Vipera russelli*, phospholipase A derived from bee venom yielded identical results. Similar dose-dependent changes were elicited by phospholipases C and D; however, greater concentrations of these enzyme preparations were required. The reduction of (−)-[^{3}H]dihydroalprenolol binding after exposure to phospholipase A was not due to solubilization of intact receptor sites. Assay of the soluble phase of the incubations by equilibrium dialysis and Sephadex G-50 chromatographic tech-

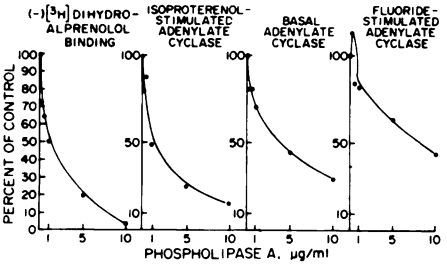

FIG. 1. *Concentration dependence of phospholipase A effects on (−)-[³H]dihydroalprenolol binding and adenylate cyclase activities in frog erythrocyte membranes*

Membranes (8.9 mg/ml) were incubated for 10 min at 25° in the absence and presence of the indicated concentrations of enzyme. Control (−)-[³H]dihydroalprenolol binding was 0.23 pmole/mg of protein. Basal adenylate cyclase activity was 55.5 pmoles of cAMP generated per minute per milligram of protein. Isoproterenol-stimulated activity was calculated as the difference in cAMP generated in the absence (basal) and presence of 0.1 mM isoproterenol. Fluoride-stimulated activity was calculated as the difference in cAMP generated in the absence (basal) and presence of 10 mM NaF. The source of phospholipase A was *Vipera russelli*. This experiment is representative of four separate experiments in which (−)-[³H]dihydroalprenolol binding was reduced 50% by 1.06 ± 0.05 μg/ml of phospholipase A and basal adenylate cyclase was reduced 50% by 4.9 ± 1.16 μg/ml of phospholipase A.

niques (9) after a 60-min centrifugation of the digested membranes at 105,000 × *g* revealed no binding activity. The effects of the phospholipase preparations were also not due to contaminating proteolytic activities. When phospholipase A preparations were tested for proteolytic activity using an extremely sensitive procedure, the ability to degrade human fibrinogen (13, 14), none was found. In addition, as expected, the simultaneous presence of 5 mM EGTA in the membrane preparations completely prevented the observed phospholipase effects, since phospholipase A has previously been shown to have an absolute requirement for Ca^{++} (15). Apparently sufficient Ca^{++} was present as a contaminant in the incubation medium (probably in the Tris buffer) to satisfy the requirement of phospholipase A for Ca^{++}.

Figure 1 also demonstrates that phospholipase A digestion of frog erythrocyte membranes decreases adenylate cyclase activity in a dose-dependent fashion. Exposure of frog erythrocyte membranes to 0.1–10 μg/ml of phospholipase A reduces basal and fluoride-stimulated adenylate cyclase activities and causes an even greater decrease in isoproterenol-stimulated adenylate cyclase. The marked decline in catecholamine responsiveness of the enzyme directly parallels the reduction in (−)-[³H]dihydroalprenolol binding.

A series of experiments was undertaken to determine whether the observed decreases in (−)-[³H]dihydroalprenolol binding were due to a decrease in receptor number or affinity, or both. The saturation binding data in Fig. 2A demonstrate that the diminished (−)-[³H]dihydroalprenolol binding observed after phospholipase A digestion of frog erythrocyte membranes is due to a reduction in the number of binding sites, with insignificant alteration in the affinity of the remaining sites. This decrease in receptor number is reflected by a decrease in V_{max} of catecholamine-stimulated adenylate cyclase activity in the absence of significant change in the concentration of isoproterenol which elicits half-maximal enzyme stimulation (Fig. 2B).

In another series of experiments we attempted to determine whether exposure of digested membranes to particular lipids could restore receptor binding or catecholamine-stimulated adenylate cyclase. Frog erythrocyte membranes were exposed to 5 μg/ml of bee venom phospholipase A for 10 min at 25°. EGTA was then added to a final concentration of 5 mM to terminate phospholipase A digestion. Membranes were then exposed to either phosphatidylserine, phosphatidylcholine, phosphatidylinositol, or a frog erythrocyte membrane total lipid extract by swirling the phospholipase A-treated preparations in vessels coated with 1 mg/ml of the particular lipid. None of these lipids had any restorative effect on catecholamine-sensitive adenylate cyclase activity (data not shown). In each of three experiments, phosphatidylserine and phosphatidylinositol significantly increased (−)-[³H]dihydroalprenolol binding in these phospholipase A-treated preparations. However, this enhanced binding activity was not stereospecific and displayed low affinity, and thus did

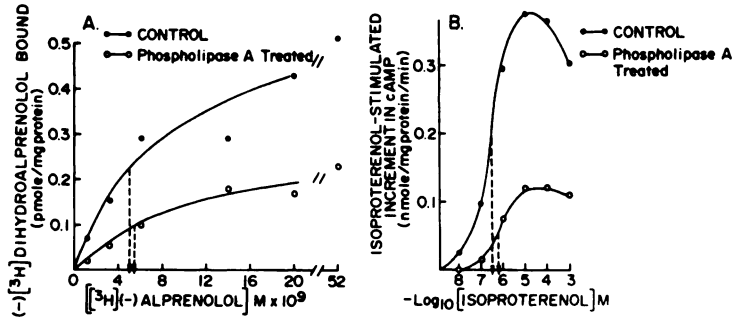

FIG. 2. *Effects of phospholipase A treatment of erythrocyte membranes*

A. Effect on (−)-[^{3}H]dihydroalprenolol binding. Membranes (6.5 mg/ml) were incubated for 10 min at 25° in the presence ("treated") or absence ("control") of 1 μg/ml of bee venom phospholipase A. The arrows (▼) indicate the concentrations of (−)-[^{3}H]dihydroalprenolol which half-maximally occupied the sites in each preparation.

B. Effect on isoproterenol-stimulated adenylate cyclase. Membrane preparations were treated with phospholipase A (6.5 mg/ml) as described for Fig. 2A. At the end of the 10-min preliminary incubation, binding and enzyme activities were assayed simultaneously. Basal adenylate cyclase activity was 74.3 pmoles of cAMP generated per minute per milligram of protein in the control preparation and 51.3 pmoles/min/mg of protein in the phospholipase-treated preparation.

not represent restoration of functional *beta* adrenergic receptor binding.

Effects of nondegradative membrane perturbants on specific (−)-[^{3}H]dihydroalprenolol binding and adenylate cyclase activities. A number of agents capable of interacting with the lipid matrix of biomembranes were tested for their effects on *beta* adrenergic receptor binding and adenylate cyclase activities. For example, amphotericin B and nystatin are polyene antibiotics which have been demonstrated to penetrate preferentially the cholesterol-containing regions of biological membranes (16, 17). Treatment of frog erythrocyte membranes with 10–500 μg/ml of amphotericin B mimics the dose-dependent effects on binding and cyclase activities observed with phospholipases (Fig. 3). Thus (−)-[^{3}H]dihydroalprenolol binding and isoproterenol-stimulated adenylate cyclase activity are diminished in a parallel fashion and to a much greater extent than basal and fluoride-stimulated activities. Nystatin, however, does not lead to dose-dependent alterations in binding and enzymatic activities when studied over the same concentration range (data not shown).

The experiments with phospholipases and the membrane-perturbing agents suggest that membrane lipids are crucially

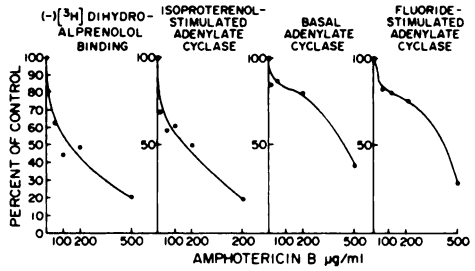

FIG. 3. *Effects of amphotericin B on (−)-[^{3}H]dihydroalprenolol binding and adenylate cyclase activities in frog erythrocyte membranes*

Amphotericin B was suspended with the membranes (6.15 mg/ml) by swirling for 10 min at 25° as described in METHODS. Control (−)-[^{3}H]dihydroalprenolol binding was 0.21 pmole/mg of membrane protein. Basal adenylate cyclase in the control preparation was 38.6 pmoles/min/mg of membrane protein. Isoproterenol-stimulated and fluoride-stimulated enzyme activities were calculated as described in Fig. 1. Results are the means of duplicate determinations, which agreed within 6%.

involved in the function of the *beta* adrenergic receptor binding sites. Several other possible interpretations of the data were considered, and rejected after appropriate experimentation. Treatment of membranes with the various perturbants might lead to the formation of lipid micelles which could interact with (−)-[^{3}H]dihydroalprenolol, thus lowering the

effective concentration of the radioligand and artifactually decreasing binding. To assess this possibility, membrane preparations were treated with each of these agents under the usual conditions. The 100,000 × *g* supernatants from these preliminary incubations, which contained the perturbants and the soluble products of their interaction with membranes, were then incubated with (−)-[^{3}H]dihydroalprenolol for 10 min at 37° to determine whether (−)-[^{3}H]dihydroalprenolol was trapped in or bound to products of these incubations. Aliquots of the supernatants were then chromatographed on Sephadex G-50 columns at 4°, conditions under which dissociation of the radiolabeled compound is negligible during the time course of the chromatography (9). In no case was significant (−)-[^{3}H]dihydroalprenolol found to be trapped or bound to soluble products derived from the membranes. In all cases (−)-[^{3}H]dihydroalprenolol added was quantitatively retrieved in the salt volume of the columns. These results indicate that the decreases in (−)-[^{3}H]dihydroalprenolol binding observed after membrane treatment with the agents was not due to sequestering of (−)-[^{3}H]dihydroalprenolol and a subsequent reduction of the effective concentration of (−)-[^{3}H]dihydroalprenolol in the binding assays.

Another possibility considered was that the observed decreases in (−)-[^{3}H]dihydroalprenolol binding following the various membrane treatments reflected aggregation of the membranes induced by these agents, with concomitant shielding of *beta* adrenergic receptor sites. First, it was demonstrated that (−)-[^{3}H]dihydroalprenolol binding was linearly related to membrane protein concentration between 0 and 7.0 mg/ml before and after treatment with each of the perturbants. Second, electron microscopy of treated membranes revealed no evidence of membrane vesicle aggregation or other changes consistent with steric hindrance of access to the cell membrane surface.

The polyene antibiotic Filipin has strikingly different effects on specific (−)-[^{3}H]dihydroalprenolol binding from those observed with the other membrane perturbants described above. Binding is not diminished by greatment of membranes with Filipin (Fig. 4), whereas catecholamine-stimulated adenylate cyclase activity is markedly diminished, to a greater extent than the reduction of basal and fluoride-stimulated activities. The binding of (−)-[^{3}H]dihydroalprenolol in the Filipin-treated membranes did not appear to be a result of nonspecific adsorption of radioligand to Filipin or altered membrane components, since the (−)-[^{3}H]dihydroalprenolol binding sites in these treated membranes retained their strict stereospecificity for the (−) isomers of *beta* adrenergic agents (data not shown).

Effects of proteolytic digestion of (−)-[^{3}H]dihydroalprenolol binding. Exposure of frog erythrocyte membranes to the proteolytic enzyme trypsin caused a decrease in specific (−)-[^{3}H]dihydroalprenolol binding (Table 1). Decreases in specific binding were due almost entirely to decreases in total radioligand binding with negligible increases in "nonspecific" binding. When erythrocyte membranes were exposed simultaneously to trypsin and phospholipase A, a synergistic effect on the diminution of (−)-[^{3}H]dihydroalprenolol binding

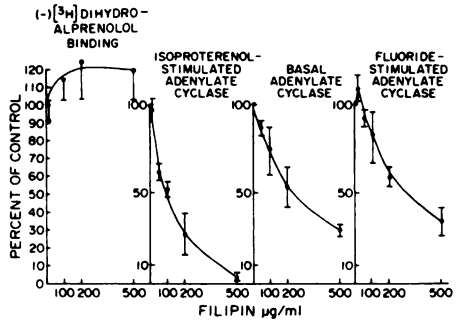

FIG. 4. *Effects of Filipin on (−)-[^{3}H]dihydroalprenolol binding and adenylate cyclase activities in frog erythrocyte membranes*

Filipin was suspended with membranes for 10 min at 25° as described in METHODS. Values shown are the means and standard errors of duplicate determinations from five separate experiments. Mean control (−)-[^{3}H]dihydroalprenolol binding was 0.30 ± 0.08 pmole/mg of membrane protein. Mean basal adenylate cyclase activity in the control preparations was 106.1 ± 30 pmoles/min/mg of protein. Membrane protein concentrations ranged from 2.7 to 6.6 mg/ml. Isoproterenol-stimulated and fluoride-stimulated enzyme activities were calculated as described in Fig. 1.

TABLE 1

Effect of enzymes on specific (−)-[³H]dihydroalprenolol binding

Membrane preparations (1.9–2.6 mg/ml) were incubated in the presence of the indicated concentrations of enzymes for 10 min at 25° in 75 mM Tris–25 mM $MgCl_2$, pH 8.1. Control preparations were incubated under identical conditions, but without enzymes. Then 100-μl aliquots were transferred to the usual incubation medium for assay of specific binding. Values are the means of duplicate determinations from two separate experiments, in which control binding was 0.153–0.160 pmole/mg of membrane protein.

Enzyme	Concentration	[³H]Dihydroalprenolol binding
	μg/ml	*% control*
Trypsin	5	31
	2	36
	1	46.7
	0.5	69.1
Combined enzymatic treatment		
Trypsin	0.5	71
Phospholipase A[a]	0.5	64
Trypsin + phospholipase A[a]	0.5 + 0.5	16

[a] Bee venom enzyme.

was observed. In fact, complete degradation of receptor binding could not be effected by trypsin without the simultaneous presence of phospholipase A.

DISCUSSION

These results suggest that membrane lipids are involved in each of the functional components of the catecholamine-sensitive adenylate cyclase system: receptor binding, enzyme catalysis, and coupling of receptor binding to adenylate cyclase stimulation. Binding to the *beta* adrenergic receptor was reduced by treatment of membranes with either degradative (e.g., phospholipases) or nondegradative (e.g., amphotericin B) lipid-perturbing reagents. The synergistic effect of trypsin and phospholipase A on degradation of binding suggests that the *beta* adrenergic receptor may be a proteolipid, since proteolipids are similarly characterized by resistance to proteolytic enzymes alone (18).

The sensitivity of adenylate cyclase to phospholipases has been demonstrated previously, and it has been concluded that these enzymes are lipoproteins (19). In addition, other investigators have reported the anomalous increase in fluoride-stimulated enzyme activity with exposure to low phospholipase concentrations (19, 20).

A role for membrane lipids in receptor-enzyme coupling is also consistent with the observations of previous investigators. For example, Rethy *et al.* (21) demonstrated that organic solvent extraction or phospholipase digestion of rat liver plasma membranes results in the loss of adenylate cyclase responsiveness to epinephrine, glucagon, and fluoride. Epinephrine-stimulated enzyme activity can be partially restored by the addition of phosphatidylserine (21). Digestion of canine cardiac membranes with phospholipase A, C, or D has been reported to decrease basal and fluoride-sensitive adenylate cyclase and abolish catecholamine-stimulated activity (22). The catecholamine responsiveness in this system could be restored only by the addition of a total lipid fraction extracted from the myocardial membranes. These observed decreases in catecholamine responsiveness following perturbation of membrane lipids, however, could be due either to a reduction in *beta* adrenergic binding or to disruption of receptor-enzyme coupling, or both.

Our studies with Filipin provide direct evidence for a role of membrane lipids in coupling *beta* adrenergic receptor binding to adenylate cyclase activation. Filipin reduced isoproterenol-stimulated adenylate cyclase to a much greater extent than basal and fluoride-stimulated activity, but this was not accompanied by any decrease in receptor binding. Thus the disproportionate loss of catecholamine-sensitive enzyme activity in the Filipin-treated preparations is due entirely to alterations distal to the *beta* adrenergic receptors. Puchwein *et al.* (23) studied in detail the manner in which Filipin interacts with a comparable model system, the pigeon erythrocyte membrane. Filipin is capable of interacting with membrane cholesterol to form a Filipin-cholesterol complex. It was observed that Filipin perturbation of the erythrocyte membrane involved regions probed with the hydrophobic dye perylene

but not those accessible to aqueous solvents probed by 8-anilino-1-napthalenesulfonic acid. Puchwein *et al.* (23) also claimed to have demonstrated an "uncoupling" effect, since catecholamine-sensitive adenylate cyclase was reduced with no effect on putative *beta* adrenergic receptor binding in their preparations. However, these investigators employed (±)-[^{3}H]epinephrine and (±)-[^{3}H]isoproterenol as radioactive tracers of receptor binding. Since tritium-labeled agonists have been found to label a number of sites unrelated to the physiologically relevant *beta* adrenergic receptor (24), our studies with (−)-[^{3}H]dihydroalprenolol are the first to validate categorically that Filipin is capable of uncoupling the catecholamine-sensitive adenylate cyclase system.

The disproportionate decrease in isoproterenol-stimulated adenylate cyclase activity caused by phospholipases A, C, and D as well as amphotericin B is also consistent with the hypothesis that membrane lipids play a role in receptor-enzyme coupling. However, the concomitant degradation of *beta* adrenergic receptor binding sites caused by these agents prevents an assessment of the extent to which the decline in catecholamine-sensitive enzyme activity is due to alterations in the receptors or to an interruption of "signal transmission."

It seems reasonable to conclude that membrane lipids are involved in both receptor binding and receptor-enzyme coupling. A question which remains to be answered is whether lipids simply maintain a membrane architecture crucial for a hormonally responsive state of the enzyme or whether specific phospholipids are responsible for transmission of a signal from receptor to enzyme. The data of Levey and Klein (25, 26) have been interpreted to indicate that specific phospholipids are involved in coupling specific hormone-receptor interactions with adenylate cyclase activation. Adenylate cyclase preparations from cat ventricular myocardium are rendered unresponsive to hormone stimulation following solubilization with Lubrol PX. Levey observed that monophosphatidylinositol specifically restored catecholamine responsiveness (25) and that phosphatidylserine specifically restored glucagon and histamine responsiveness (26) of adenylate cyclase in these solubilized preparations. However, no binding studies were undertaken to determine the functional status of the *beta* adrenergic receptors in these preparations. Thus the lipid effects observed could possibly represent restoration of the ability to bind catecholamines rather than to couple receptor binding to enzyme activation.

In contrast to the observation of Levey, the observations of Rethy *et al.* (21) and Lefkowitz (22) described above suggest much less specificity for particular phospholipids in restoring catecholamine sensitivity in solvent- and phospholipase-treated membrane preparations. We found that neither total lipid fractions nor particular phospholipids were able to restore stereospecific *beta* adrenergic receptor binding or catecholamine-sensitive adenylate cyclase activity in phospholipase A-treated frog erythrocyte membranes.

It is apparent that the ability to study directly both *beta* adrenergic receptor binding and adenylate cyclase activity in the present experiments provides more complete insight into the loci of perturbation effects caused by disruption of the lipid matrix of biological membranes.

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