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# Functional reconstitution of β-adrenergic receptors and the stimulatory GTP-binding protein of adenylate cyclase

(hormone action/guanine nucleotides)

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**ABSTRACT** **A procedure for the functional reconstitution of β-adrenergic receptors and the stimulatory guanine nucleotide-binding protein (G/F) of adenylate cyclase in phospholipid vesicles is described. β-Adrenergic receptors were solubilized from turkey erythrocyte plasma membranes and reconstituted into phospholipid vesicles by the addition of dimyristoyl phosphatidylcholine and removal of detergent by gel filtration. This procedure restored the ability to bind [$^{125}$I]iodohydroxybenzylpindolol and [$^{3}$H]dihydroalprenolol. Purified rabbit hepatic G/F that was added to the receptor vesicles could be stably activated by guanosine 5′-[3-thio]triphosphate at a low rate, and this activation was increased up to 4-fold in the presence of β-adrenergic agonists. This stimulation of the activation of G/F was specific for β-adrenergic agonists and could be specifically blocked by β-adrenergic antagonists. Stimulation was proportional to the concentration of vesicles containing active β-adrenergic receptor. Under optimal conditions, 5 to 6 molecules of G/F were activated per receptor, indicating that catalytic activation of G/F by receptor was reconstituted.**

Hormone-sensitive adenylate cyclase is composed of at least two protein components in addition to receptors for hormones: the catalytic unit and a guanine nucleotide-binding regulatory protein (G/F) (1). Stimulation of adenylate cyclase activity by fluoride and guanine nucleotides is mediated by G/F and is not a direct action on the catalytic protein. Hormonal stimulation, for which β-adrenergic stimulation is prototypical, is mediated via two distinct protein–protein interactions that can be considered to be sequential. First, β-adrenergic receptors with bound hormone interact with G/F, in the presence of guanine nucleotide, to produce an activated state of G/F. The activated G/F then interacts with the catalyst to stimulate its activity. The primary control point for the stimulation of adenylate cyclase activity by β-adrenergic agonist and guanine nucleotides is the receptor–G/F interaction (1).

Little definitive information is available on the molecular mechanism of the receptor–G/F interaction. It can take place only when both proteins are bound to a membrane, and the composition and structure of the membrane probably influence the coupling of receptor and G/F. Incorporation of the β-adrenergic receptor into a phospholipid bilayer also determines many of its ligand-binding activities (2). Therefore, this system is best studied in a reconstituted preparation that allows the interaction of purified receptors with G/F in a well-defined and easily manipulated phospholipid environment.

Citri and Schramm (3) recently developed a reconstituted system that combined crude, detergent-solubilized G/F and β-adrenergic receptors from turkey erythrocytes with soy phospholipids. In their preparation, isoproterenol stimulated the stable activation of G/F by Gpp(NH)p.

We report here the development and use of a similar reconstituted system for the study of the receptor–G/F interaction. Because of our use of purified G/F, a simpler protocol for the reconstitution of receptors prior to the addition of G/F, and a simpler and more direct assay for activated G/F, we think that this system is more amenable to detailed quantitative analysis. It also allows independent manipulation of the concentration of each protein as well as of the bilayer composition. This report demonstrates the β-adrenergic catalysis of the activation of purified hepatic G/F by guanosine 5′-[3-thio]triphosphate (GTPγS) in vesicles composed primarily of dimyristoyl phosphatidylcholine ($Myr_2$PtdCho). The data also suggest functional consequences of the stoichiometric relationships of receptors and G/F in individual vesicles.

## EXPERIMENTAL PROCEDURES

**Materials.** $Myr_2$PtdCho, obtained from Sigma, was dispersed in 10 mM Tris·HCl/0.1 mM EDTA, pH 8.0, to a concentration of 50 mg/ml by using a bath type sonicator. Cholic acid (Sigma) was purified by DEAE-cellulose chromatography (4). Deoxycholic acid (Sigma, grade II) was recrystallized from ethanol/water, 1:1 (vol/vol). [α-$^{32}$P]ATP was prepared according to Johnson and Walseth (5). GDPβS and GTPγS were purchased from Boehringer Mannheim, isomers of propranolol were a gift from Ayerst (New York), phentolamine was a gift from Sterling–Winthrop (Rensselaer, NY), and isomers of isoproterenol were purchased from Sigma.

Plasma membranes from cyc$^-$ S49 lymphoma cells were prepared as described (6). [$^{125}$I]Iodohydroxybenzylpindolol (IHYP) was prepared as described by Maguire *et al.* (7). G/F was purified from rabbit liver as described by Sternweis *et al.* (8) with the following exceptions. $AlCl_3$ (10 μM) was substituted for ATP in all elution buffers (9). Chromatography on hydroxyapatite was omitted. The eluate from heptylamine-agarose was applied to DEAE-Sephacel and the column was washed with 2 vol of 0.1% Lubrol 12A9/25 mM NaCl/20 mM Tris·HCl/1 mM EDTA/1 mM dithiothreitol, pH 8.0. G/F was eluted with the same buffer containing 250 mM NaCl.

**Preparation and Assay of Vesicles Containing β-Adrenergic Receptors.** Unless otherwise indicated, all manipulations were carried out at 0–4°C. Washed, packed, turkey erythrocytes were diluted with 5 vol of 20 mM Tris·HCl, pH 7.5/1.0 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride (lysis buffer); then excess phenylmethylsulfonyl fluoride was added to 0.6 mM final concentration. The mixture was centrifuged for 15 min at 10,000 rpm in a Beckman JA-10 rotor. Pellets were removed

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Abbreviations: G/F, stimulatory GTP-binding protein of adenylate cyclase; GTPγS, guanosine 5′-[3-thio]triphosphate; IHYP, iodohydroxybenzylpindolol; $Myr_2$PtdCho, dimyristoyl phosphatidylcholine.

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in a minimal volume of lysis buffer and homogenized with 12–14 strokes of a loose-fitting Dounce homogenizer. The homogenate was diluted with 1 vol of lysis buffer and centrifuged for 15 min at 2,500 rpm in a JA-10 rotor. Membranes remaining in the supernatant were repeatedly washed with lysis buffer until the supernatant was colorless. This procedure yields about 12 g of membrane protein from 32 liters of turkey blood.

β-Adrenergic receptors were solubilized from turkey erythrocyte membranes by a modification of the procedure of Eimerl *et al.* (10). Membranes were centrifuged and resuspended to 2 mg of protein per ml in 20 mM NaHepes, pH 8.0/8 mM $MgCl_2$/1.0 mM EDTA/1 mM 2-mercaptoethanol/0.1 mM ascorbate/20 μM (−)-isoproterenol and incubated for 15 min at 30°C. After cooling to 0°C, the membranes were centrifuged and resuspended in 20 mM NaHepes, pH 8.0/1.0 mM EDTA/1.0 M NaCl/2 mM $MgCl_2$ to 2 mg of protein per ml after the addition of deoxycholate. Deoxycholate (10% solution) was added slowly with stirring to a final concentration of 0.6%. After a 10-min stirring period at 0°C, insoluble material was removed by centrifugation at 50,000 rpm for 1 hr in a Beckman 70 Ti rotor.

To reconstitute solubilized receptors, the extract was supplemented with $Myr_2$PtdCho to a concentration of 1 mg/ml. This mixture was chromatographed on Sephadex G-50 in 20 mM NaHepes, pH 8.0/0.2 mM EDTA/0.1 M NaCl at a flow rate of 0.4 ml/min. The sample volume was less than 10% of the bed volume. Turbid fractions in the void volume that contained [$^{125}$I]IHYP binding activity were pooled and concentrated by centrifugation through a layer of 12.5% (wt/vol) sucrose onto a layer of 50% sucrose (usually 4 hr at 40,000 rpm in a SW 41 Ti rotor). The material at the 12.5%–50% sucrose interface was divided into small portions and frozen at −80°C. This preparation is referred to as "receptor vesicles."

Rabbit hepatic G/F was reconstituted with β-adrenergic receptor vesicles by addition of the purified G/F to a dilute suspension of vesicles. Routinely, final concentrations were 0.1 M NaCl, 20 mM NaHepes (pH 8.0), 2 mM $MgCl_2$, 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.1 mM ascorbate, 0.25 mg of vesicle protein per ml (0.25–0.50 nM β-adrenergic receptors), and 6 μg of G/F per ml. The suspension of vesicles plus G/F was held on ice 20 min prior to activation. To measure the activation of G/F in vesicles, adrenergic agents or excess $MgCl_2$ was added and the mixture was equilibrated at 30°C for 1 min. GTPγS (10 μM, final concentration) was then added to initiate activation, which was continued at 30°C. The activation was terminated by dilution with 6.5 vol of a chilled quenching solution, yielding final concentrations of 0.1% Lubrol 12A9, 0.1 mM GDPβS, 0.1 mM (−)-propranolol, 100 mM NaCl, 0.1 mM ascorbate, 20 mM NaHepes (pH 8.0), 5 mM EDTA, and 1 mM 2-mercaptoethanol. GDPβS does not activate G/F (11) but blocks binding of GTPγS; EDTA chelates $Mg^{2+}$, which can promote activation of G/F (8); propranolol blocks the action of any residual β-adrenergic agonist; and Lubrol promotes subsequent reconstitution of G/F with the catalytic protein of adenylate cyclase (8). Quenching is essentially immediate according to a comparison of samples that were quenched at zero time with samples to which GTPγS was added after quenching or to which no GTPγS was added.

Activated G/F produced in the incubation was assayed on the basis of its ability to activate the catalytic component of adenylate cyclase (8). Nonactivated G/F is without effect in this assay. Catalytic protein for the assay of activated G/F was provided by plasma membranes of cyc$^-$ S49 lymphoma cells which lack G/F (8, 12). For assay, a 30-μl aliquot of quenched activation mixture was mixed with 30 μl of cyc$^-$ plasma membranes (4 mg/ml) and held at 0°C for at least 10 min. The adenylate cyclase assay was initiated by addition of 40 μl of assay medium to yield 0.5 mM [α-$^{32}$P]ATP (20–40 cpm/pmol), 7 mM $MgCl_2$, 0.1 mg of bovine serum albumin per ml, 1 mM EDTA, 10 μg of pyruvate kinase per ml, 6 mM dipotassium phospho*enol*pyruvate, 0.2 mM 1-methyl-3-isobutylxanthine, 50 mM NaHepes (pH 8.0), 0.1 mM GDPβS, 0.1 mM (−)-propranolol, and 0.1 mM ascorbate. The assay was carried out for 10 min at 30°C, at which time cyclic [$^{32}$P]AMP was isolated according to Salomon *et al.* (13). This assay is linear with activated G/F over a wide range of total G/F concentrations. One unit of G/F activity is defined as the stimulation of 1 nmol/min of adenylate cyclase activity in this assay (8).

**Other Assays.** Binding of [$^{125}$I]IHYP to β-adrenergic receptors was measured essentially as described (2). [$^{125}$I]IHYP was incubated with vesicles or membranes for 60 min at 30°C in 20 mM NaHepes, pH 8.0/12 mM $MgCl_2$/1 mM EDTA. Bound ligand was separated from free by filtration on Whatman GF/F filters. Protein was assayed as described by Schaffner and Weissman (14). Lipid phosphorus was determined as described by Ames (15) after digestion with $HClO_4$ at 180°C.

## RESULTS

**Reconstitution of Vesicle-Bound Receptors.** Solubilization of β-adrenergic receptors by deoxycholate resulted in the loss of assayable [$^{125}$I]IHYP-binding activity in the assay described above or of [$^3$H]dihydroalprenolol binding as measured by the gel filtration assay of Fleming and Ross (2). The binding activity was restored by chromatography of the $Myr_2$PtdCho-supplemented extract on Sephadex G-50; at least 60%, and usually 80%, of the [$^{125}$I]IHYP-binding activity originally in the membranes was recovered in the eluate. This material bound at least 1.0 pmol of [$^{125}$I]IHYP per mg of protein, at most a 4-fold purification relative to plasma membranes. In this preparation, referred to as receptor vesicles in analogy to previous studies from this laboratory (2), $Myr_2$PtdCho accounted for ≈60% of the total phospholipid. The remainder was residual phospholipid from the erythrocyte membranes. Vesicles usually contained 2 mg of phospholipid per mg of protein and <0.5% of the deoxycholate applied to the column. Preincubation of the erythrocyte membranes with (−)-isoproterenol prior to solubilization with deoxycholate was necessary to restore >20% of the original IHYP-binding activity of the membranes, and propranolol could not substitute for isoproterenol. GTP or Gpp(NH)p at 0.05 mM did not alter the stabilizing effect of isoproterenol, suggesting that a stable receptor–G/F–agonist complex is not the species that is solubilized (1, 16).

Binding of [$^{125}$I]IHYP to receptor vesicles was consistent with the existence of a single class of binding sites. The $K_d$s for [$^{125}$I]IHYP and other β-adrenergic ligands were slightly higher in the vesicles than in native membranes, as noted previously (2), but characteristic stereoselectivity and rank order of affinity were maintained (Table 1). Each unlabeled ligand displaced at saturation the same amount of [$^{125}$I]IHYP, defining a level of nonspecific binding that routinely was <20% of the total [$^{125}$I]IHYP bound in the absence of competitor.

**Reconstitution of Receptor–G/F Interaction.** Purified rabbit hepatic G/F that had been reconstituted with receptor vesicles was stably activated by GTPγS at a low rate in the presence of 1 mM free $Mg^{2+}$ (2 mM total $MgCl_2$). However, the rate of activation was markedly stimulated by the addition of (−)-isoproterenol (Fig. 1). The stimulation was transitory and was essentially complete by 8 min at 30°C, as shown by comparison of the rates of activation in the presence of isoproterenol and of isoproterenol plus propranolol. The magnitude of the stimulation at short times usually was 3- to 4-fold and occasionally was greater (Table 2).

The data of Table 2 and Fig. 2 imply that the increase in the

Table 1. Equilibrium dissociation constants for binding of β-adrenergic ligands in reconstituted vesicles and in membranes

| Ligand | $K_d$, nM | |
|---|---|---|
| | Vesicles | Erythrocyte membranes |
| [$^{125}$I](±)IHYP | 0.026 | 0.037 |
| (−)-Isoproterenol | 12 | 250 |
| (+)-Isoproterenol | 10,000 | 150,000 |
| (−)-Propranolol | 1.5 | 5 |
| (+)-Propranolol | 120 | 1,500 |

$K_d$ for [$^{125}$I]IHYP was determined as described (2). To determine $K_d$s for unlabeled ligands, receptor vesicles (0.6 μg) or erythrocyte membranes (1.2 μg) were incubated with 26 or 40 pM [$^{125}$I]IHYP plus varying concentrations of competing ligand. [$^{125}$I]IHYP binding was determined, and $K_d$ was calculated by multiplying the $IC_{50}$ for each ligand by the fraction of unoccupied receptors when no competing ligand was added (7).

rate of activation of G/F is mediated by the reconstituted β-adrenergic receptors. Stimulation was blocked by propranolol, a β-adrenergic antagonist, but not by phentolamine, an α-adrenergic antagonist. Terbutaline, a noncatecholamine agonist, also stimulated. Antagonists alone had no effect but generally were included when basal activation rates were measured in case any isoproterenol was carried over during preparation of the vesicles. Stereoselectivity for the isomers of isoproterenol was identical with respect to competition for [$^{125}$I]IHYP binding and to the stimulation of activation of G/F. The potency of propranolol isomers to inhibit the isoproterenol-stimulated activation of G/F also corresponded well to their affinity for vesicle-bound receptors—(−) isomer, $IC_{50}$ = 0.52 nM, $K_d$ = 1.5 nM; (+) isomer, $IC_{50}$ = 170 nm, $K_d$ = 120 nM. Also shown in Table 2 are data from control incubations to which hepatic G/F was not added. These data indicate that residual turkey erythrocyte G/F does not contribute significantly to the amount of activated G/F that is measured in these experiments.

**Quantitation of Receptor–G/F Coupling.** The extent of the β-adrenergic stimulation of activation represents ≈10% of the total G/F present. This total was estimated as the amount of G/F that was activated after 15 min at 30°C in the presence of 50 mM $Mg^{2+}$, conditions that completely activate G/F (ref. 8; confirmed by us). The low fraction of G/F that can be activated in a hormone-specific process does not reflect the denaturation of G/F or β-adrenergic receptor during the initial incubation

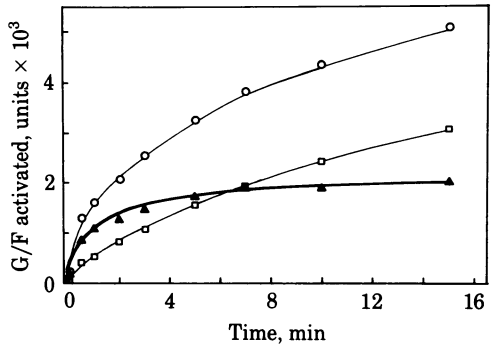


FIG. 1. Receptor-stimulated activation of G/F in phospholipid vesicles. Receptor vesicles and G/F were reconstituted to final concentrations of 0.25 mg/ml of vesicle protein and 6 μg/ml of G/F. G/F activation was carried out at 30°C in the presence of (−)-isoproterenol (1.0 μM) (○) or (−)-isoproterenol plus propranolol (10 μM) (□). At the times indicated, 10 μl aliquots were removed and added to 65 μl of quenching solution. The increase in activation due to (−)-isoproterenol is also shown (▲).

Table 2. β-Adrenergic specificity of the activation of G/F

| Addition | G/F activated, units × $10^3$ | |
|---|---|---|
| | Lubrol control | Hepatic G/F added |
| None | 0.01 | 0.7 |
| (−)-Isoproterenol (1.0 μM) | 0.02 | 2.9 |
| (−)-Propranolol (10 μM) | 0.03 | 0.7 |
| (−)-Isoproterenol and propranolol | 0.02 | 0.8 |
| Terbutaline (0.1 mM) | 0.18 | 2.1 |
| Terbutaline and propranolol | 0.03 | 0.7 |
| Phentolamine (10 μM) | 0.04 | 0.8 |
| (−)-Isoproterenol and phentolamine | 0.03 | 2.7 |
| $MgCl_2$ (50 mM) | 0.10 | 8.7 |

Receptor vesicles were reconstituted with G/F or with 0.1% Lubrol/20 mM NaHepes/1 mM EDTA. Activation was carried out in the presence of the indicated ligands for 1 min after addition of GTPγS.

at 30°C. [$^{125}$I]IHYP binding activity after the incubation was at least 85% of that of a sample held at 0°C. These receptors were still capable of interacting with G/F because addition of more

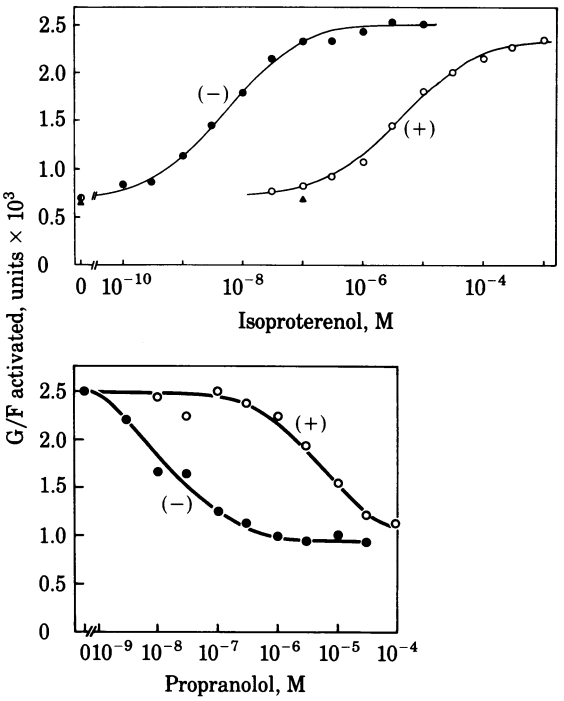


FIG. 2. Stereoselectivity for agonists and antagonists for the activation of G/F in receptor vesicles. Reconstitution of G/F and receptor vesicles was carried out as described in Fig. 1. (*Upper*) G/F reconstituted with vesicles was activated in the presence of varying concentrations of (−)-isoproterenol (●) or (+)-isoproterenol (○). The activation incubation was stopped 1 min after addition of GTPγS. Activities in the presence of 10 μM propranolol and the indicated concentrations of (−)-isoproterenol are also shown (▲). Half-maximal stimulation occurred at 7 nM (−)-isoproterenol and 7,000 nM (+)-isoproterenol. (*Lower*) G/F reconstituted with vesicles was activated in the presence of 0.3 μM (−)-isoproterenol and varying concentrations of (−)-propranolol (●) or (+)-propranolol (○). Activation was stopped 3 min after addition of GTPγS. Half-maximal inhibition occurred at 15 nM (−)-propranolol and 5,000 nM (+)-propranolol. To compare these values to the $K_d$s for each isomer (Table 1), they can be adjusted according to the concentration of isoproterenol that was present by dividing them by ([isoproterenol]/$EC_{50}$) + 1 (see ref. 7). The corrected $IC_{50}$ values are 0.5 nM and 170 nm for the (−) and (+) isomers of propranolol. Note also the relative increase in the basal activation rate due to the 3-min activation time.

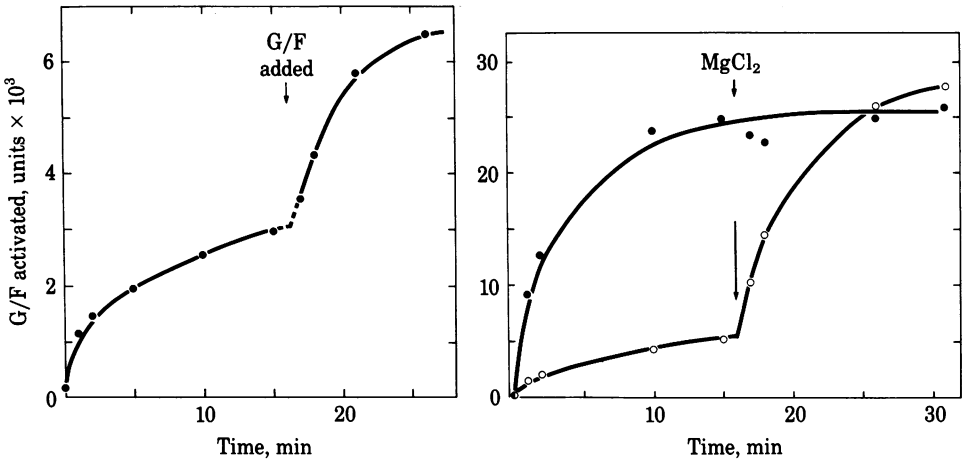


FIG. 3. Stability of β-adrenergic receptors and G/F during the activation process. (*Left*) Receptor vesicles and G/F were reconstituted and G/F was activated as in Fig. 1. After 16 min, an amount of G/F was added equal to that added initially (6 μg/ml; added in 0.02 vol). Activation was measured in the presence of 1 μM (−)-isoproterenol and of (−)-isoproterenol plus 10 μM propranolol, and the difference between the two samples is shown. (*Right*) Receptor vesicles and G/F were reconstituted and G/F was activated in the presence of 50 mM $MgCl_2$ (●) or 1 μM (−)-isoproterenol (○). Aliquots were taken at the indicated times for assay of activated G/F. After 16 min, 1 M $MgCl_2$ was added to each incubation mixture to increase the concentration by 50 mM.

G/F after 15 min of incubation resulted in further receptor-mediated stimulation of activation (Fig. 3 *Left*). In this experiment the amount of G/F added at 16 min was equal to that added originally, and the second burst of stimulation was as great as the first. The data of Fig. 3 *Right* demonstrate that G/F also was not significantly lost during the 15-min activation assay because the addition of 50 mM $MgCl_2$ produced the same amount of GTPγS-activated G/F whether it was added before or after the initial 15 min. The addition of more vesicles at 15 min had no effect on the activation of G/F.

The magnitude of the β-adrenergic stimulation of G/F activation was proportional to the number of receptors present in the vesicles, and there was no stimulation by agonist in the absence of active receptors (Fig. 4). This titration of receptors was performed at a constant concentration of G/F, protein, and phospholipids by mixing receptor vesicles that contained active receptors with vesicles that contained denatured receptors. The increase in the number of active receptors used in this experiment had no effect on the maximal amount of G/F that could be activated by 50 mM $Mg^{2+}$ (data not shown). Thus, the fraction of G/F activation that was receptor-mediated also increased linearly with receptor number. The slope of the isoproterenol-stimulated activation in Fig. 4 can be used as a measure of the number of G/F molecules activated per β-adrenergic receptor. This calculation assumes a molecular weight for G/F of 80,000 (8) and a specific activity of 15,000 units/mg of protein (17). Using these values, we calculate that 1.1 molecules of G/F were activated per receptor in this experiment. However, the conditions used in this experiment are suboptimal for G/F activation. Higher absolute levels of specific β-adrenergic stimulation of activation were observed at longer times (Fig. 1) or when more G/F was added (Figs. 3 *Left* and 5). Calculations from data obtained at longer times and higher G/F concentrations indicate that 5 to 6 molecules of G/F have

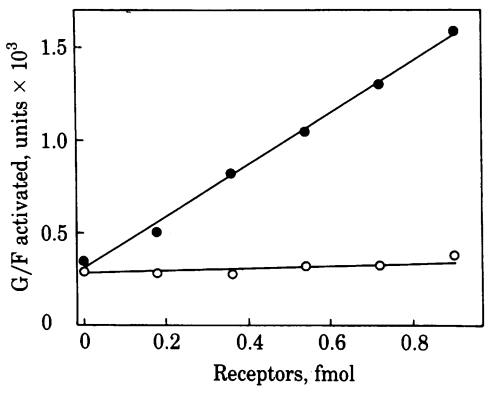


FIG. 4. Stimulation of activation of G/F by isoproterenol depends on β-adrenergic receptors. Vesicles that contained insignificant [$^{125}$I]IHYP binding activity (<50 fmol/mg; <5% of receptor-containing vesicles) were prepared by the procedure used to prepare receptor vesicles, except that isoproterenol was omitted from the 30°C incubation of membranes prior to solubilization and the deoxycholate extract was warmed at 30°C for 20 min. These vesicles were similar to receptor vesicles in terms of lipid/protein ratio and the yield of protein. Receptor vesicles and vesicles that contained denatured receptors were mixed and each mixture was reconstituted with G/F. G/F was activated for 1 min in the presence of 1 μM (−)-isoproterenol (●) or (−)-isoproterenol plus 10 μM propranolol (○). The number of receptors shown on the abscissa is the amount ultimately transferred to a single G/F assay mixture. This number should be multiplied by 250 to yield the picomolar concentration of receptors in the activation.

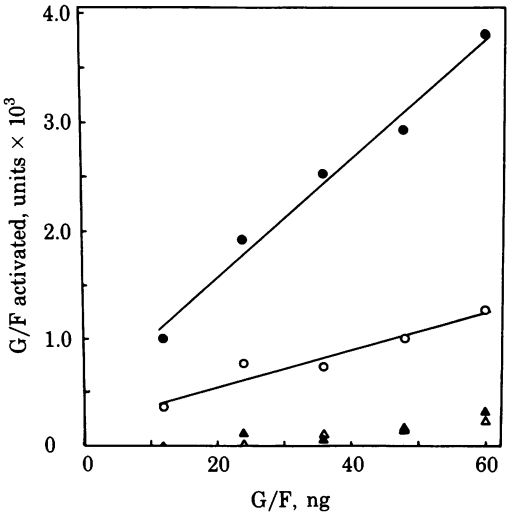


FIG. 5. The amount of G/F that is activated is proportional to the amount added to the activation incubation. G/F was diluted with 0.1% Lubrol/20 mM Hepes, pH 8.0/ 1 mM EDTA and then diluted 1:20 into the vesicle suspension. This procedure introduces 2.5-fold more Lubrol into the reconstituted system than in the standard procedure but has no apparent effect on the extent or rate of G/F activation. The amount of G/F activated during a 1-min incubation in the presence of 1 μM (−)-isoproterenol (●) or (−)-isoproterenol plus 10 μM propranolol (○) was measured. The amount of G/F shown is that in each 0.1-ml assay mixture. It can be multiplied by 250 to yield the concentration of G/F (in ng/ml) in the activation mixture.

been activated per receptor, a value we still consider to be submaximal.

Both the initial β-adrenergic stimulation of activation (1 min) and the extent of this activation (15 min) increased linearly with the amount of G/F that was added to a constant amount of vesicles (Fig. 5; only 1-min data are shown). In this experiment, the extent of β-adrenergic stimulation at 15 min was 5 molecules of G/F activated per receptor. The maximal amount of G/F that was activated, determined by using 50 mM $MgCl_2$, was also proportional to the amount that was added. Thus, the fraction of G/F that underwent receptor-stimulated activation was constant as a function of the amount of G/F added. Therefore, receptors are not saturated with respect to their ability to activate G/F and are consistent with receptor-mediated catalysis of the activation of G/F by GTPγS.

## DISCUSSION

The data presented here demonstrate the functional reconstitution of β-adrenergic receptors and G/F from distinct sources in a phospholipid environment that is predominantly $Myr_2$-PtdCho. Reconstitution of the receptors restores both their ability to bind [$^{125}$I]IHYP or [$^3$H]dihydroalprenolol and, more importantly, their ability to facilitate the activation of G/F by guanine nucleotides. The procedure described here is similar in principle to that described by Citri and Schramm (3), but it offers several significant advantages. The use of purified rabbit hepatic G/F and the assay for activated G/F are probably the most important. Having a purified, concentrated preparation of G/F allows us to control its concentration over a wide range without introducing significant quantities of detergent [the phospholipid/detergent ratio typically is 25:1 (wt/wt)]. The receptor-to-G/F ratio is thus manipulated easily. The assay for G/F is simple, quantitative, and reproducible, involving only the addition of the quenched reaction mixture to cyc$^-$ membranes and a subsequent adenylate cyclase assay. A negative aspect of hepatic G/F compared with turkey erythrocyte G/F is its significant rate of activation by GTPγS in the absence of hormone, causing a less-impressive relative stimulation (compare refs. 8 and 18). Finally, the receptor reconstitution reported here can be carried out with good yield on a large scale (250 mg) and may be adapted to alter the lipid composition of the vesicles. An important criterion of receptor function is the ability to activate multiple molecules of G/F catalytically (1). Because the molecular weight and specific activity of hepatic G/F are known, we can calculate that reconstituted receptors can catalyze the activation of at least 6 G/F molecules (see *Results*). This value is conservative and is a strong argument that reconstituted receptors display their physiological function.

The receptor-catalyzed activation of G/F in the vesicles system is about 10% of the total. This apparently low efficiency and the short duration of receptor action are not due to significant loss of either receptors or G/F during activation (Fig. 3) and may simply reflect the distribution of few receptors among many vesicles rather than intrinsically poor coupling. If the receptor vesicles are 1,000 Å in diameter and if receptors and G/F are randomly distributed among them, then only 10% of the vesicles contain a single β-adrenergic receptor and each vesicle contains 5–10 molecules of G/F. (See ref. 19 for the calculation of vesicle concentration.) If only 10% of the vesicles contain receptors and if only those G/F molecules that are on receptor-bearing vesicles can be catalytically activated, then the coupling of receptors and G/F may be excellent in those vesicles. This explanation is consistent with the experiment of Fig. 4 in which both the net amount of G/F and the fraction of total G/F that were catalytically activated were proportional to the concentration of receptor vesicles. The inability of vesicle-bound G/F to exchange among vesicles is suggested by the lack of effect of adding excess receptor vesicles to vesicles already reconstituted with G/F. A more detailed critique of this argument must await physical characterization of the vesicles. The argument may also be tested by altering the size of the vesicles and by using more highly purified receptors to prepare vesicles that contain an average of more than one receptor each.

The major physiological function of adenylate cyclase-linked hormone receptors is to catalyze the activation of G/F by guanine nucleotides, and this function is restored by the reconstitution presented here. In preliminary experiments, we have also reproduced the characteristic negatively cooperative binding interaction of guanine nucleotides and β-adrenergic agonists (1). In the absence of added G/F, Gpp(NH)p has no effect on the affinity of vesicle-bound receptors for (−)-isoproterenol. When G/F was added, however, Gpp(NH)p caused a 2.5-fold decrease in the $K_d$ for this agonist (unpublished data). Restoration of both of these aspects of receptor–G/F coupling suggests that the receptor vesicles are a valid experimental system and should be useful for the study of receptor–G/F coupling and the role of the membrane bilayer in that process.

**Note Added in Proof.** Electron micrographs of negatively stained receptor vesicles indicate unilamellar vesicles of typical diameter 1,000 Å (range, 500–3,000 Å).

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