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Biochemistry

Solubilization of human platelet α-adrenergic receptors: Evidence that agonist occupancy of the receptor stabilizes receptor–effector interactions

(catecholamines/adenylate cyclase/agonist versus antagonist)

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ABSTRACT **The α-adrenergic receptors of human platelet membranes can be directly identified by both a radiolabeled agonist, [^{3}H]epinephrine, and a radiolabeled antagonist, [^{3}H]yohimbine. Digitonin solubilizes a binding component from the membrane that is indistinguishable from the α-receptor identified in the native platelet membrane as assessed by (*i*) order of potency of agonists and antagonists and (*ii*) affinity of the receptor for [^{3}H]-yohimbine and competing antagonists. However, the solubilized receptor demonstrates a reduced affinity for agonists and a loss of the ability of guanine nucleotides to modulate receptor affinity for agonists. Prelabeling of human platelet membranes with [^{3}H]-epinephrine results in a guanine nucleotide-sensitive agonist–receptor complex that sediments more rapidly in sucrose gradients than do unoccupied or antagonist-occupied receptors. Thus, agonist occupancy of the α-receptor prior to membrane solubilization may promote or stabilize receptor interaction with effector components in the membrane, one of which may be the GTP regulatory protein responsible for modulation of receptor affinity.**

The functional coupling of receptor occupancy to adenylate cyclase activation involves at least three separate macromolecules in the target membrane: (*i*) the specific receptor responsible for recognition of the hormone or drug, (*ii*) a catalytic subunit that catalyzes the conversion of ATP to cyclic AMP, and (*iii*) a guanine nucleotide regulatory protein (G protein) that mediates the effects of GTP on the expression of catalytic activity and on modulation of receptor affinity for agonists (1). Thus, the G protein(s) appears to play a pivotal role in transducing receptor binding by hormones or agonist drugs into enzyme activation.

Much less is known about the molecular components or events involved in the transduction of receptor occupancy into attenuation of adenylate cyclase. However, the known requirements and characteristics of attenuating systems as tabulated by Jakobs (2) suggest the existence of similar effector components in activating and inhibiting systems because (*i*) GTP is absolutely required for both activation and attenuation (2, 3) of adenylate cyclase and (*ii*) GTP decreases receptor affinity for agonist but not antagonist agents at receptors coupled to activation and attenuation of cyclase activity.

This report describes the direct identification of α-adrenergic receptors in human platelet membranes and successful solubilization of a binding component indistinguishable in its recognition properties from the α-receptor of native membranes but, nonetheless, insensitive to the regulatory effects of guanine nucleotides on receptor–agonist interactions. Agonist occupancy of the receptor prior to detergent solubilization appears to stabilize receptor interactions with membrane effector components, one of which may be the GTP binding protein that modulates receptor affinity for α-adrenergic agonists.

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MATERIALS AND METHODS

[^{3}H]Yohimbine (81 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and (−)-[^{3}H]epinephrine (37.3–42.8 Ci/mmol) were obtained from New England Nuclear. Digitonin was from Baker. (+)-Epinephrine and phentolamine were gifts from Sterling–Winthrop (Rensselaer, NY) and CIBA–Geigy, respectively. All other compounds were from Sigma.

Fresh platelet-rich plasma was adjusted to pH 6.5 with citric acid/citrate/dextrose (4). Washed platelet lysates were prepared as described (5) and resuspended in a Teflon/glass homogenizer into 75 mM Tris·HCl/12.5 mM $MgCl_2$/1.5 mM EDTA, pH 7.65. Protein was determined by the method of Lowry *et al.* (6). Details of methodology for each experiment are given in the figure legends.

RESULTS

Fig. 1 compares the steady-state characteristics of [^{3}H]yohimbine binding to the native membranes with those observed for binding to digitonin-solubilized preparations. Binding of [^{3}H]yohimbine was saturable, and the linear Scatchard transforms of the steady-state binding isotherms (Fig. 1 *A* and *D*) suggested a single class of binding sites in both native membranes and solubilized preparations. The order of potency of agonist and antagonist competition for [^{3}H]yohimbine binding indicated that [^{3}H]yohimbine labels platelet binding sites that exhibit a specificity characteristic of the α_2-subtype of adrenergic receptors* (Fig. 1 *B* and *C*) (8).

That the interaction of antagonists with the platelet α-receptors obeyed simple mass-action law was suggested not only by the linearity of the Scatchard transform for [^{3}H]yohimbine

Abbreviations: p[NH]ppG, 5′-guanylylimidodiphosphate, a hydrolysis-resistant analog of GTP; p[NH]ppA, 5′-adenylyl imidodiphosphate; G protein, guanine nucleotide-binding regulatory protein; EC_{50}, concentration giving 50% maximum effectiveness; *n*, number of experiments.

* Although the human platelet α-adrenergic receptor possesses agonist and antagonist specificity more closely resembling the α_2-subtype of α-adrenergic receptors when compared with the properties of the α_1-receptors (8), the platelet α-receptor does differ in certain properties from the α_2-receptors defined in the central nervous system, kidney, and other target organs. For example, whether or not clonidine, the archetypal α_2-agonist, and phenylephrine behave as agonist or antagonist agents in mediating platelet aggregation is still controversial (9, 10). Nonetheless, because phenylephrine is typically considered an agonist at α-adrenergic receptors, data obtained in competition-binding studies with this agent were plotted with agonist drugs.

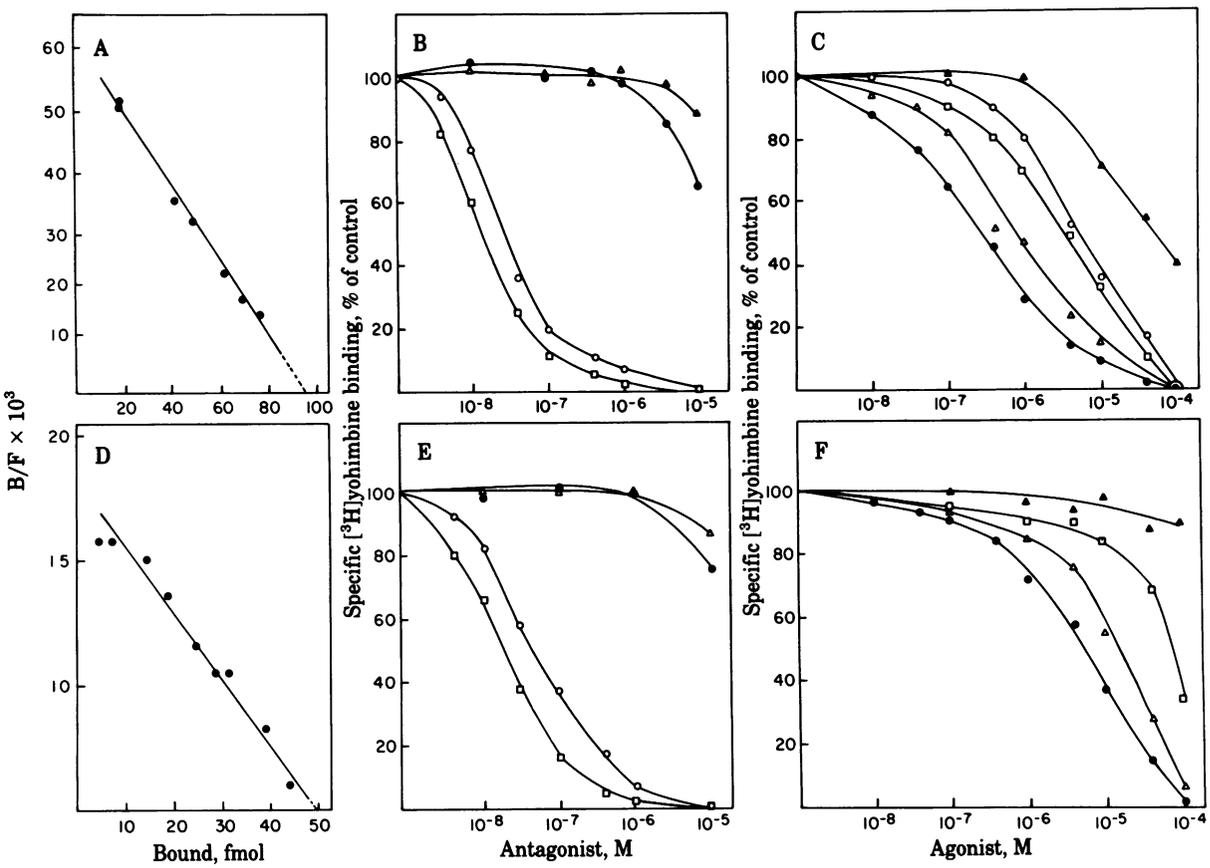

FIG. 1. Binding of [^{3}H]yohimbine to human platelet membranes (*A–C*) and digitonin-solubilized preparations (*D–F*). Scatchard analysis gave a K_d = 5.7 ± 0.4 nM and a B_{max} = 265 ± 12 fmol/mg (n = 6) for binding to membranes (Fig. 1*A*) and K_d = 7.3 ± 0.6 nM and B_{max} = 265 ± 27 fmol/mg (n = 3) for binding to solubilized preparations (Fig. 1*D*). The estimation of B_{max} (fmol/mg of protein) in solubilized preparations is somewhat inaccurate because the determination of protein concentration in these samples is complicated by the nonuniform interference of free and protein-bound digitonin with the Lowry assay. K_d values for antagonist competition for [^{3}H]yohimbine binding were calculated from concentrations giving 50% maximal effectiveness (EC_{50} values) by the method of Cheng and Prusoff (7). In membrane preparations (Fig. 1*B*) these were: yohimbine, 6.4 ± 1.7 nM (n = 6); phentolamine, 13.3 ± 0.2 nM (n = 6). ○, Phentolamine; □, yohimbine; △, prazosin; ●, propranolol. In solubilized preparations (Fig. 1*E*) K_d values were: yohimbine, 10.9 ± 1.4 nM (n = 4); phentolamine, 19.3 ± 5.7 nM (n = 3). ○, Phentolamine; □, yohimbine, ●, prazosin; △, propranolol. Because agonist competition curves were shallow, indicating deviation from simple mass-action law, calculation of the K_d for agonists from EC_{50} values was not appropriate. EC_{50} values (mean ± SEM) for membrane binding were (Fig. 1*C*): (−)-epinephrine (●), 0.28 ± 0.04 μM (n = 14); (−)-norepinephrine (△), 1.0 ± 0.03 μM (n = 5); (+)-epinephrine (□), 3.2 ± 0.79 μM (n = 3); phenylephrine (○), 5.0 ± 0.54 μM (n = 2); and isoproterenol (▲), 150 ± 2 μM. EC_{50} values for binding to solubilized preparations were (Fig. 1*F*): (−)-epinephrine (●), 6.25 ± 0.63 μM (n = 10); (−)-norepinephrine (△), 17.3 ± 3.4 μM (n = 3); and (+)-epinephrine, 68.3 ± 22.8 μM (n = 3). ▲, (−)-Isoproterenol.

Membranes were incubated with increasing concentrations of [^{3}H]yohimbine for 30 min at 30°C in a 250-μl volume containing 50 mM Tris·HCl (pH 7.65), 10 mM $MgCl_2$, 1.2 mM EDTA, and 0.3–0.5 mg of membrane protein. Incubations were terminated by vacuum filtration and washed three times with 5 ml of ice-cold buffer. Unoccupied receptors from platelet membrane pellets (≈17–25 mg of protein) were solubilized in a Teflon/glass homogenizer into 16–22 ml of 0.7% digitonin/50 mM Tris·HCl/15 mM $MgCl_2$/5 mM EDTA, which was stirred on ice for 30 min. The yield of receptors in the supernatant obtained at 105,000 × g (60 min) ("solubilized preparation") was calculated as 65% based on [^{3}H]yohimbine prelabeling and 85% based on [^{3}H]yohimbine binding to unoccupied receptors. Receptor binding in solubilized preparations was assayed after exchange into 0.1% digitonin-containing buffers with Sephadex G-50 chromatography on 1 × 22 cm columns. [^{3}H]Yohimbine (≈7.5 nM) was incubated with the Sephadex G-50 void volumes in the presence or absence of competing agents in a final volume of 0.5 ml. After the 90-min, 15°C incubation, separation of receptor-bound ligand from free ligand was accomplished at 4°C by chromatography on 0.6 × 14 cm Sephadex G-50 columns equilibrated and eluted with 0.025% digitonin/75 mM Tris·HCl/12.5 mM $MgCl_2$/1.5 mM EDTA, pH 7.65. The 0.9-ml volume corresponding to the elution volume for blue dextran 2000 ("bound") was collected and counted in 10 ml of Triton X-100-toluene scintillation fluor. Specific binding (not competed for by 10 μM phentolamine) was <75% of total binding.

binding (Fig. 1*A*) but also by the normal steepness† of the ^{3}H-labeled antagonist/antagonist curves (Fig. 1*B*). In contrast to ^{3}H-labeled antagonist/antagonist competition curves, the ^{3}H-labeled antagonist/agonist competition curves were shallow† (Fig. 1*C*). Recent studies utilizing computer modeling of shallow [^{3}H]dihydroergocryptine antagonist/agonist competition curves have indicated the existence of interconvertible high- and low-affinity states for agonist binding to human platelet α-adrenergic receptors (12, 13).

Exposure of human platelet membranes to the plant glycoside digitonin solubilized a binding site which retained its recognition properties for adrenergic agents. The solubilized binding site possessed an affinity for [^{3}H]yohimbine [K_d = 7.3 ± 0.6 nM; number of experiments (n) = 3] virtually identical with

† The term "normal steepness" refers to the shape of a competition curve which proceeds from 90% → 10% competition over an 81-fold concentration range of competitor. This is the behavior expected of a competitor that interacts with the receptor through a reversible, bimolecular reaction obeying simple mass-action law and that competes for a radioligand that also meets the same restrictions (11). "Shallow" curves extend over a greater than 81-fold concentration range of competitor and are consistent with negatively cooperative interactions or receptor heterogeneity. Certain technical problems can also give rise to shallow competition curves, including inappropriate or dissimilar definitions of nonspecific binding for agonist and antagonist competitors or an incubation duration too brief to allow the lowest concentration of competitor to reach steady state. The possibility of an artifactual appearance of shallow curves for agonist competition when compared with antagonist competition was evaluated for the present studies and found not to pertain.

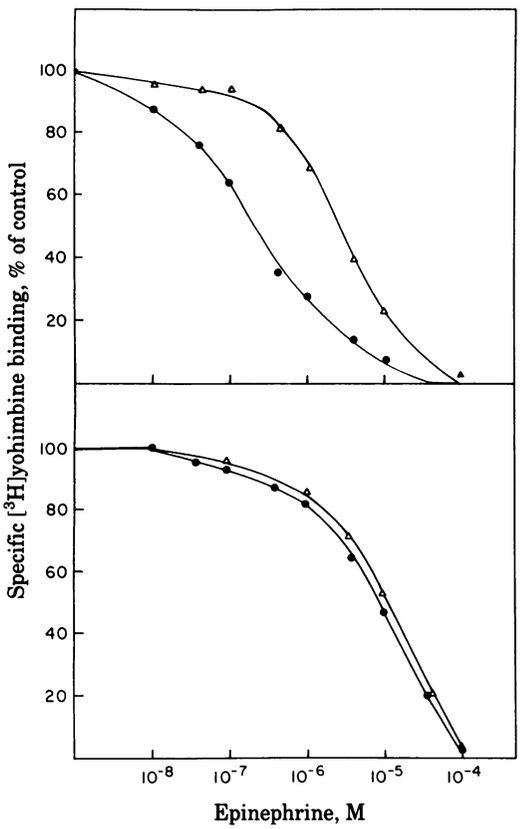

FIG. 2. Effect of guanine nucleotides on agonist competition for [^{3}H]yohimbine binding to membrane (*A*) and digitonin-solubilized (*B*) preparations. The EC_{50} (mean ± SEM) for (−)-epinephrine competition in membrane preparations was 0.28 ± 0.04 μM (n = 14) in the absence (●) and 2.57 ± 0.27 μM (n = 9) in the presence (△) of 0.1 mM p[NH]ppG (*A*). In solubilized preparations, (*B*), the EC_{50} for (−)-epinephrine was 6.25 ± 0.63 μM (n = 10) in the absence (●) and 9.56 ± 1.02 μM (n = 9) in the presence (△) of 0.1 mM p[NH]ppG (*B*). The specificity for this effect is p[NH]ppG > GTP ≥ GDP >> GMP; p[NH]ppA has no effect ≤ 1 mM. Receptor–antagonist interactions were not modified by guanine nucleotides in either membrane or solubilized preparations.

that observed in membranes (K_d = 5.7 ± 0.4 nM; n = 6). In addition, antagonists demonstrated an identical specificity and affinity in competing for [^{3}H]yohimbine binding in solubilized preparations (Fig. 1*E*) as in native membranes (Fig. 1*B*). Agonists also competed for [^{3}H]yohimbine binding to solubilized preparations with the same order of potency as that observed in native membranes. However, the EC_{50} values for agonists were considerably increased after solubilization, indicating a selective loss in receptor affinity for agonists upon membrane disruption (Fig. 1*F*).

The hypothesis that solubilization may disrupt receptor interactions with membrane components involved in conferring a higher affinity of the receptor for agonists is consistent with the observation that guanine nucleotides did not modulate receptor affinity for agonists in solubilized preparations in which the receptors were unoccupied at the time of solubilization (Fig. 2*B*). This is in distinct contrast to native membranes, wherein p[NH]ppG both increased the EC_{50} of (−)epinephrine competition for [^{3}H]yohimbine binding and increased the steepness of the competition curve (Fig. 2*A*).

To determine whether agonist occupancy of platelet membrane receptors can stabilize receptor interactions with effector components conferring sensitivity to guanine nucleotides, we first identified human platelet α-receptors with the radiolabeled agonist, [^{3}H]epinephrine (Fig. 3). The calculated K_d values observed for antagonist competition for [^{3}H]epinephrine binding (Fig. 3*A*) were comparable to those values obtained when antagonists competed for [^{3}H]yohimbine binding (Fig. 1*B*). However, EC_{50} values for agonist competition for [^{3}H]epinephrine binding were approximately a factor of 100 lower than those observed when agonists competed for [^{3}H]yohimbine binding (Fig. 1*C*). Furthermore, the K_d for (−)-[^{3}H]epinephrine binding obtained from Scatchard analysis (Fig. 4*A*) was 1.8 ± 0.8 nM (n = 3). The data in Figs. 3*B* and 4*A* thus suggest that binding detected using 5–7 nM [^{3}H]epinephrine represents binding to the high-affinity state of the receptor for agonists. Fig. 4*B* indicates that guanine nucleotides facilitated the rate of [^{3}H]epinephrine dissociation from platelet membranes, demonstrating that the high-affinity state of the α-receptor identified by [^{3}H]epinephrine is sensitive to guanine nucleotides.

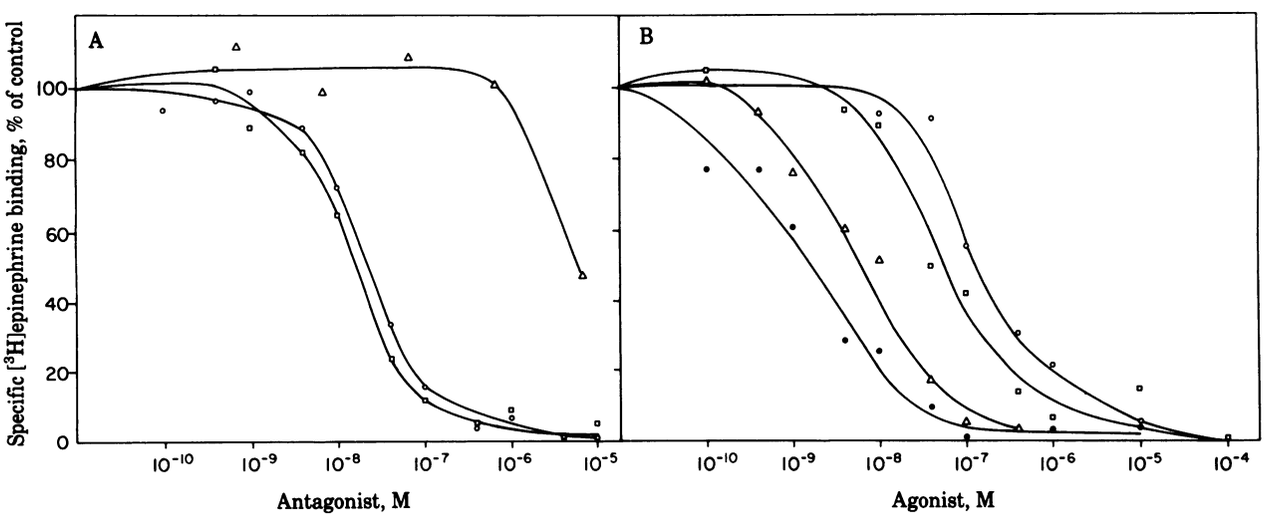

FIG. 3. Competition of adrenergic antagonists (*A*) and agonists (*B*) for [^{3}H]epinephrine binding to human platelet membranes. Incubations were for 2 hr at 15°C in 1.0 ml containing 5–6 nM [^{3}H]epinephrine, 1.0 mM catechol, 0.1% ascorbic acid, 1 μM pargyline (to prevent ligand degradation by monoamine oxidase), 1 μM propranolol (to block α-adrenergic receptors), and 0.1–0.3 mg of membrane protein and were terminated as in Fig. 1. Specific binding (not competed for by 10 μM phentolamine) was > 60% of total binding. Data points are the mean values of duplicate determinations in 2–5 separate experiments. Calculated (9) K_d values for antagonists were: yohimbine (□), 4.3 ± 0.7 nM (n = 2), and phentolamine (○), 6.3 ± 1.2 nM (n = 2). △, Prazosin. EC_{50} values for agonists (mean ± SEM) were: (−)-epinephrine (●), 3.3 ± 1.2 nM (n = 5); (−)-norepinephrine (△), 7.3 ± 2.8 nM (n = 4); (+)-epinephrine (□), 37.5 ± 7.8 nM (n = 4); and phenylephrine (○), 150 nM (n = 2).

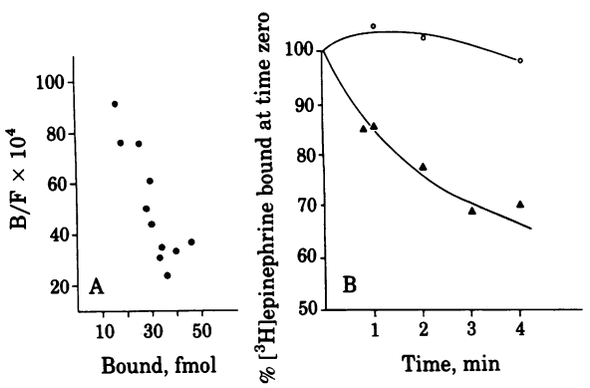

FIG. 4. (*A*) Scatchard transform of [^{3}H]epinephrine binding to human platelet membranes. The data are from one experiment representative of three separate experiments where apparent K_d, calculated from the slope of the steepest asymptote to the data points, was 1.8 ± 0.8 nM. The scatter in the data apparent at concentrations of [^{3}H]epinephrine >16 nM (B, >30 fmol) probably results from [^{3}H]epinephrine interaction with a "second site," presumably the "low-affinity state" of the α-receptor for agonist agents. (*B*) Dissociation of [^{3}H]epinephrine from human platelet α-adrenergic receptors. Membranes (0.175 mg/ml) were incubated with 6.7 nM [^{3}H]epinephrine for 2 hr at 15°C. Dissociation was initiated by adding 10 μM phentolamine in the absence (○) or presence (▲) of 0.1 mM p[NH]ppG; 1-ml aliquots were taken at the times indicated. When dissociation is studied over longer periods, the rate constant k_2 for [^{3}H]epinephrine dissociation in the presence of phentolamine can be calculated, equaling ≈0.017 ± 0.0045 min^{-1} (n = 4). The association rate constant k_1, estimated in separate experiments, equaled ≈2.62 × 10^{-6} M^{-1} min^{-1}. The kinetically calculated $K_d = k_2/k_1 \approx 4.08$ nM is in reasonable agreement with the K_d determined in steady-state binding experiments (1.8 ± 0.8 nM) (*A*).

To assess whether agonist occupancy of the receptor might indeed stabilize receptor interactions with the effector component(s) that confer sensitivity to guanine nucleotides, we prelabeled membranes with [^{3}H]epinephrine prior to digitonin solubilization. The rate of [^{3}H]epinephrine dissociation from the solubilized [^{3}H]epinephrine–receptor complex was assessed in the presence and absence of p[NH]ppG. As shown in Fig. 5, p[NH]ppG facilitated the dissociation of [^{3}H]epinephrine from the prelabeled, solubilized agonist–receptor complex in a manner analogous to observations in intact membranes (Fig. 4) but in contrast to observations for receptors solubilized from the membrane in an unoccupied state (Fig. 2).

To evaluate whether the apparent ability of agonist occupancy of human platelet α-adrenergic receptors to stabilize receptor–effector interactions might also be manifested by alterations in the molecular properties of solubilized receptors, [^{3}H]epinephrine–receptor and [^{3}H]yohimbine–receptor complexes were subjected to sucrose gradient centrifugation subsequent to solubilization. [^{3}H]Epinephrine–receptor complexes sedimented more rapidly than either [^{3}H]yohimbine antagonist–receptor complexes or unoccupied receptors (Fig. 6). Because the specificity of agonists and antagonists in competing for both [^{3}H]yohimbine (Fig. 1) and [^{3}H]epinephrine (Fig. 3) binding was identical, it reasonably could be concluded that both ligands were binding to the same recognition site in the target membrane. Thus, the different sedimentation profiles of prelabeled [^{3}H]epinephrine– and [^{3}H]yohimbine–receptor complexes did not reflect binding to independent populations of recognition sites. Several phenomena might account for the faster sedimentation of agonist-occupied receptors, including (*i*) a change in receptor shape, causing a decrease in its frictional ratio; (*ii*) a change in receptor conformation, resulting in increased binding of the detergent digitonin, which possesses a partial specific volume similar to that of proteins; or (*iii*) an increase in protein mass of the agonist–receptor complex due to agonist-stabilized association of the receptor with additional membrane components.

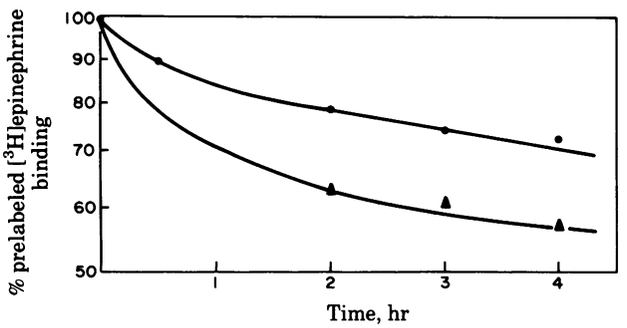

FIG. 5. Guanine nucleotide-facilitated dissociation of [^{3}H]epinephrine from the digitonin-solubilized α-adrenergic receptor. Human platelet membranes were incubated with 7 nM [^{3}H]epinephrine as in Fig. 3 and solubilized with digitonin as in Fig. 1. The rate of [^{3}H]epinephrine dissociation from the prelabeled, solubilized receptors was determined at 15°C in the absence (●) and presence (▲) of p[NH]ppG (1 mM). The amount remaining bound at each time point was determined by subjecting 0.5-ml aliquots to Sephadex G-50 chromatography (4°C) on the 0.6 × 14 cm columns. [^{3}H]Epinephrine binding at time zero was 900 cpm/0.5-ml aliquot of the solubilized preparation.

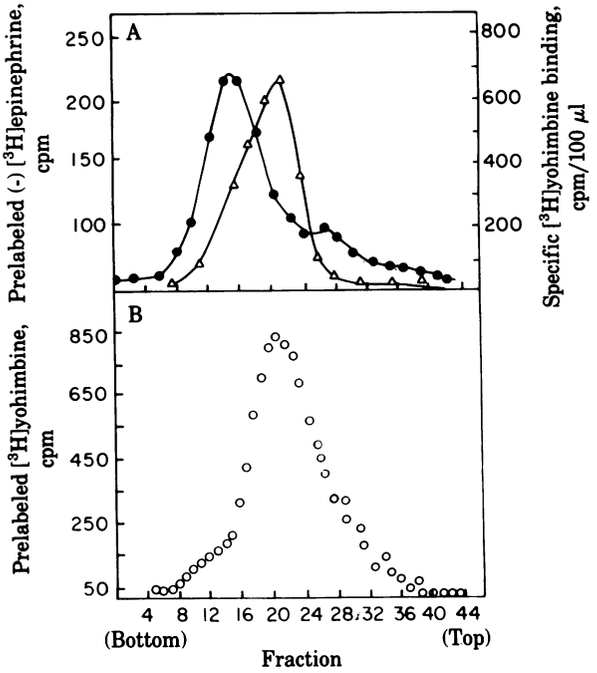

FIG. 6. α-Adrenergic receptor of human platelet. Comparison of the sedimentation characteristics of [^{3}H]epinephrine-prelabeled α-receptors (●) (*A*), [^{3}H]yohimbine-prelabeled α-receptors (○) (*B*), and unoccupied receptors (△) (*A*) on 5–20% sucrose gradients. Prelabeling of membranes was with 6 nM [^{3}H]epinephrine or 7.5 nM [^{3}H]yohimbine. The digitonin-solubilized preparation was applied to 1× 22 cm Sephadex G-50 columns equilibrated with 0.1% digitonin to remove "free" radioligand and excess detergent prior to concentration with Amicon C-25 Centriflo cones. Centrifugation was for 15 hr through 12-ml gradients containing 0.1% digitonin buffer. Gradients were collected from the bottom in 10-drop fractions (≈0.25 ml). Dissociated [^{3}H]yohimbine, which occasionally appears in fractions 26–50, can be resolved from protein-bound ligand by Sephadex G-50 chromatography subsequent to fractionation, as was done here. Unoccupied receptors (*A*) were assayed in the gradient fractions with 15 nM [^{3}H]yohimbine (△) (see Fig. 1 legend). The sedimentation properties of unoccupied or [^{3}H]yohimbine-occupied receptors are not modified if the membranes are incubated under conditions identical to those employed for prelabeling with [^{3}H]epinephrine (see Fig. 1 legend and Fig. 3). Sample recovery was >90% from the gradients. The sedimentation coefficient for agonist-prelabeled α-receptors is ≈13.4 and for unoccupied receptors is ≈11.4, based on comparison with internal ^{14}C-labeled protein markers.

Although we cannot unequivocally rule out the possibility that a change in shape or detergent binding occurred, we favor the interpretation that the change in sedimentation behavior of the receptor in the presence of agonist is due, at least in part, to association of the receptor with membrane components conferring sensitivity to guanine nucleotides. First, Fig. 5 shows that guanine nucleotide sensitivity was manifested by the solubilized [^{3}H]epinephrine–receptor complex created prior to detergent exposure but not by epinephrine–receptor complexes generated subsequent to detergent solubilization (Fig. 2). Second, prelabeling of platelet membranes with 6 nM [^{3}H]epinephrine in the presence of p[NH]ppG or GTP prevented the appearance of the faster sedimenting form, consistent with the ability of GTP to decrease receptor affinity for agonist (Fig. 2*A*) and, thus, to limit the detectability of [^{3}H]epinephrine–receptor complexes associated in the presence of 6 nM [^{3}H]epinephrine. Finally, incubation of membranes with 0.1 mM p[NH]ppG in the presence of 40 nM [^{3}H]epinephrine appeared to reverse or prevent formation of the faster sedimenting form of the α-receptor. Under these conditions, the [^{3}H]epinephrine–receptor complex sedimented in the region characteristic of unoccupied receptors but with a broader sedimentation profile, an effect presumably due to [^{3}H]epinephrine dissociation during sedimentation from the guanine-nucleotide-promoted lower-affinity form of the receptor (data not shown). Taken together, these findings suggest that the agonist-promoted increase in sedimentation rate of the α-adrenergic receptor is due, at least in part, to receptor association with effector components, one of which is probably the GTP-binding regulatory protein modulating receptor affinity for agonist.

DISCUSSION

Several observations indicate that receptor occupancy by agonists but not antagonists promotes or stabilizes a high-affinity, guanine-nucleotide-sensitive state of the receptor. First, agonist competition curves for [^{3}H]yohimbine binding are shallow. Computer analysis of similarly shallow curves for (−)-epinephrine competition for [^{3}H]dihydroergocryptine binding to platelet α-receptors has been interpreted to represent an agonist-promoted increase in the affinity of a fraction of the receptor population (12). The guanine nucleotide sensitivity of this high-affinity state for agonists is manifested both by the increased EC_{50} observed when agonists compete for [^{3}H]yohimbine binding in the presence of 0.1 mM p[NH]ppG (Fig. 2*A*) and by the p[NH]ppG-facilitated dissociation of [^{3}H]epinephrine binding (Fig. 4*B*). Antagonists, in contrast, do not demonstrate shallow curves in competing for [^{3}H]yohimbine binding, and their interaction with the receptor is not modulated by guanine nucleotides. A second difference between receptor–agonist and receptor–antagonist interactions is the ability of agonist-occupancy of the human platelet α-adrenergic receptor to promote or stabilize receptor–effector interactions which are demonstrable subsequent to membrane solubilization. Thus, agonist occupancy of the α-receptors prior to solubilization promotes formation of a guanine nucleotide-sensitive receptor–ligand complex (Fig. 5), which sediments more rapidly than antagonist-occupied or unoccupied receptors (Fig. 6).

A number of the phenomena uniquely promoted by agonist occupancy of human platelet α-receptors are reminiscent of observations in receptor systems coupled to activation, rather than attenuation, of adenylate cyclase. For example, agonist occupancy of membrane-bound β-adrenergic receptors promotes formation of a higher-affinity receptor–agonist complex that is modulated by guanine nucleotides (14–16). The higher-affinity receptor–agonist interaction is thought to represent a ternary complex between the agonist, the receptor, and the G protein (16). Consistent with this interpretation is the observation that agonist but not antagonist occupancy of frog erythrocyte and rat reticulocyte β-receptors promotes formation of a receptor–G protein complex, isolated based on its larger molecular size (17, 18). Formation of this complex is prevented or reversed by guanine nucleotides (18). Recent studies have demonstrated that this agonist-promoted β-receptor–G protein complex is an obligatory first step in catecholamine activation of adenylate cyclase (19–21) and, furthermore, that the extent to which an agonist promotes or stabilizes the ternary complex correlates with the extent to which catecholamines can amplify catalytic activity (22). The phenomenological similarity of hormonal systems coupled to activation and attenuation of adenylate cyclase poses the question of whether or not a single population of G proteins conveys both activating and inhibiting signals to the catalytic component of the adenylate cyclase system. The apparent ability of agonist occupancy of human platelet α-receptors to stabilize receptor interactions with other membrane components should provide the appropriate starting material for biochemical and reconstitution studies aimed at resolving the composition of the agonist-promoted receptor complex and the relationship of its components to those involved in activation of adenylate cyclase.

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