*Proc. Natl. Acad. Sci. USA*
Vol. 77, No. 11, pp. 6401–6405, November 1980
Biochemistry

# Cytoskeletal constraint of the β-adrenergic receptor in frog erythrocyte membranes

(propranolol/microtubules/adenylate cyclase/fluorescence/catecholamines)

BRUCE D. CHERKSEY*†, JOSE A. ZADUNAISKY†, AND RANDALL B. MURPHY*

*Department of Chemistry, New York University, 4 Washington Place, New York, New York 10003; and †Department of Physiology and Biophysics, and the Department of Ophthalmology, New York University School of Medicine, 550 First Avenue, New York, New York 10016

*Communicated by W. F. Libby‡, July 25, 1980*

**ABSTRACT** A fluorescence receptor binding assay, based upon the high-affinity β-adrenergic receptor antagonist propranolol, is utilized to probe the microenvironment of the antagonist-receptor complex in the frog (*Rana catesbeiana*) erythrocyte membrane. The technique of steady-state fluorescence depolarization is applied to the propranolol-receptor complex, allowing quantitation of the rotational relaxation time of the complex. It is found that the complex is dynamically constrained at 20°C. However, in the temperature range 6–10°C a sharp reversible release of constraint is observed. It is further demonstrated that the addition of drugs that are known to specifically disrupt the cytoskeleton (colchicine, vincristine, and vinblastine) causes a similar but irreversible release of constraint at 20°C. Cytochalasin B has a much smaller influence on the rotational mobility of the propranolol-receptor complex than do the other drugs that disrupt the cytoskeleton. Amphotericin B is without effect on the rotational constraint of the complex. Binding of the antagonist [$^3$H]dihydroalprenolol is not influenced by colchicine. A model is proposed which postulates that cytoskeletal elements are linked to the antagonist-receptor complex. Antagonist binding does not result in cytoskeletal release, whereas agonist binding is postulated to lead to dissociation of the agonist-receptor complex from the cytoskeleton, thereby activating adenylate cyclase.

The importance of the β-adrenergic receptor system derives from its ubiquitous distribution. The β-receptor is functionally involved in regulation of processes as diverse as epithelial chloride transport (1) and central nervous system activity (2). It has long been clear that the β-adrenergic receptor operates through an associated adenylate cyclase, which activity the receptor modulates (3). The molecular mechanism of the receptor-cyclase linkage has been the subject of considerable speculation, and although a number of hypotheses have been advanced, none have been validated or repudiated. Consequently, it is of considerable interest to employ receptor-specific biophysical probes, which allow clarification of the intimate details of the linkage.

Propranolol, 1-(isopropylamino)-3-naphthyloxy-2-propanol, is a well-characterized antagonist for the β-adrenergic receptor, to which it binds with high affinity (4–6). Its availability as a tritium-labeled compound has made possible its utilization in the characterization of the receptor.

The naphthalene nucleus of propranolol makes it a likely fluorescent substrate for the β-adrenergic receptor. Many compounds of this general structural type have been applied in the past as fluorescent probes in biological systems (7), and propranolol itself has been shown to fluoresce. This property has in fact been utilized in various pharmacokinetic studies (8, 9). We have previously shown (10, 11) that the propranolol fluorescence is sufficiently intense to allow useful determination of ligand concentration in the picomolar region. In frog (*Rana catesbeiana*) erythrocyte membrane fragments, a preparation that has been demonstrated (10–12) to contain a high density of β-adrenoceptors, added propranolol yields two emission maxima, at 340 and 356 nm. We have demonstrated (10, 11) that these two components correspond respectively to free and bound propranolol. This was determined by displacement with the (−) isomer of isoproterenol as well as by direct comparison with radiobinding assays utilizing [$^3$H]propranolol and [$^3$H]-dihydroalprenolol. Both the dissociation constant ($K_d$) of [$^3$H]propranolol from the frog erythrocyte receptor and the number of binding sites ($B_{max}$) were found to be identical for the radiobinding and fluorescence assays, within the experimental limits of confidence. Displacement studies for the (+) and (−) isomers of isoproterenol yielded identical results for both assays (10). The biologically inactive (+) isomer displaced little (5%) of the bound propranolol in either assay, whereas the active (−) isomer displaced propranolol with a typical S-shaped log dose-response curve. From these curves the concentration at which propranolol binding was 50% inhibited ($IC_{50}$) was determined to be 0.5 μM in both assays, a value in agreement with published results (12).

The agreement of the data obtained by the fluorescence binding and radiobinding assays demonstrates that the fluorescence method provides information on propranolol specifically associated with the β-adrenergic receptor at low (<5 pM) drug concentrations. The present study utilizes the fluorescence signal of the propranolol-receptor complex to probe the microenvironment of the frog β-adrenergic receptor, applying the method of steady-state fluorescence depolarization (13). Although the rotational relaxation and microviscosity of fluorescence probes that are nonspecifically located in the cellular plasma membrane have been examined, few studies have dealt directly with fluorescence probes that are specifically associated with a single defined membrane constituent. Experiments of this latter variety are of importance in view of recent data (14, 15) that raise serious questions as to the validity of interpreting the dynamic properties of individual membrane proteins from studies using nonspecific fluorescence probes. The membrane is a highly heterogeneous environment, and localized interactions clearly play an important part in defining its dynamic properties. An individual region of the membrane may behave in a manner distinct from that of the whole. Furthermore, cytoskeletal linkage of individual membrane proteins could impart dynamic properties to those constituents that isolate them from the bulk membrane lipid environment (16).

These questions concerning the microenvironment of an individual receptor population are addressed in the present study.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

‡ *Deceased, Sept. 8, 1980.*

## MATERIALS AND METHODS

**Materials.** (±)-Propranolol, as the hydrochloride, was obtained from Sigma. The drug was stated to be of greater than 99% purity. This was corroborated by thin-layer chromatography on silica gel G (E. Merck), in an ethyl acetate/hexane (75:25, vol/vol) solvent system. The propranolol as the free amine, obtained by treating the hydrochloride at 1 mg/ml with 1.0 M $Na_2CO_3$, was observed to be chromatographically homogenous, and no fluorescent impurities could be determined.

Cytochalasin B from Aldrich was kindly donated by F. Aull. Vincristine and vinblastine were obtained from Eli Lilly through standard clinical sources. Amphotericin B (Fungizone) was from Squibb. All other chemicals and buffers were of reagent grade.

**Preparation of Frog Erythrocyte Membrane Fragments.** Bullfrogs (*Rana catesbeiana*) were anesthesized with tricane methanesulfonate (Ayerst, New York) added to their bathing water. After induction of anesthesia, heparinized blood was obtained by cardiac puncture. Erythrocytes were separated at $1200 \times g$ for 10 min. The supernatant and buffy coat were removed and discarded, and the cells were resuspended in the following modified Ringer's solution (henceforth referred to as solution A): 72.5 mM NaCl, 25.0 mM $NaHCO_3$, 2.5 mM $NaH_2PO_4$, 0.7 mM $Na_2HPO_4$, 0.6 mM $Na_2SO_4$, 2.5 mM KCl, 1.2 mM $MgSO_4$, 10.0 mM sucrose, and 0.25 mM EDTA. The cells were recentrifuged at $1500 \times g$ for 10 min and the supernatant was discarded. This procedure was repeated four times.

Lysis of the erythrocytes was carried out by using a protocol (10) that has been demonstrated to yield membrane fragments essentially free of nuclear and cytoplasmic constituents. Cells were suspended in a hypotonic medium (solution B) that contained: 0.25 mM sucrose, 1.0 mM $MgCl_2$, and 5.0 mM Tris·HCl. The pH was adjusted to 7.50. The cells were agitated at 4°C for 30 min and centrifuged at $16,000 \times g$ for 20 min, and the supernatant was discarded. This procedure was repeated with solution B until a pellet essentially free of hemoglobin was obtained. This pellet was then resuspended in solution A and was utilized in this form for the fluorescence studies. The protein concentration of this homogenate was determined by using the method of Lowry *et al.* (17) to be in the range 0.05–0.10 mg/ml.

**Fluorescence Binding Assays.** A previously described (10, 11) procedure was followed. To 1.00 ml of a stock solution containing the membrane fragments was added 1.00-ml portions of propranolol in solution A, at concentrations of the latter from $1 \times 10^{-12}$ M to $1 \times 10^{-8}$ M. Assay tubes were incubated at 26°C for 15 min. At the end of this time, the contents of each tube were placed into a 1.00-cm quartz fluorescence cell, and the emission spectrum and the steady-state depolarization were quantitated. Additionally, background fluorescence of the cellular membrane fragment suspension was obtained, and correction was made for this effect. It was found that correction for the inner filter effect (18) was not necessary, provided that the total absorbance in the cuvette was maintained below 0.4 at the excitation maximum of propranolol. Above concentrations corresponding to this absorbance the inner filter effect became significant, as predicted (18).

Fluorescence spectra were obtained on a Hitachi–Perkin Elmer MPF-2A spectrofluorometer equipped with an R-818 photomultiplier. High voltage was supplied by an auxillary regulated power supply, and output could be quantitated externally on a fast picoammeter (Keithley Instruments, Cleveland, OH). These minor modifications increased the normal sensitivity of the instrument in the spectral region of interest by approximately 100 times.

Steady-state fluorescence depolarization was examined with the aid of ultraviolet transmitting Polaroid filters mounted in the emission and excitation beams. Polarization corrections for the monochromator were carried out with a solution of *N*-phenyl-1-naphthylamine in Spectrograde isooctane (19). Polarization spectra were corrected for scattering by examination of serial dilutions of erythrocyte membrane fragment suspensions and extrapolation of all observed depolarizations to infinite dilution.

The limiting polarization of propranolol was determined by examination of the depolarization of the drug in glycerol/water solutions at 26°C, at a concentration of 1 μM. The observed polarizations were extrapolated to infinite viscosity by utilizing standard methods (20). This procedure yielded a value for the limiting polarization $P_0$ of propranolol of 0.448.

**Binding Assays.** [$^3$H]Dihydroalprenolol of specific activity 55.7 Ci/mmol (New England Nuclear; 1 Ci = $3.7 \times 10^{10}$ becquerels) was utilized as a ligand for the β-adrenergic receptor due to its availability in high specific activity. [$^3$H]Propranolol of specific activity 2.761 Ci/mmol (New England Nuclear) was utilized to examine the propranolol binding as a function of temperature. In either case, 1.00 ml of the stock solution containing the membrane fragments, along with any other drugs being examined, was added to 1.00-ml samples of $^3$H-labeled ligand, in quadruplicate, at concentrations from 0.1 to 20 nM in solution A. Assay tubes were incubated at 26°C for 15 min. At the end of this time, the contents of each tube were filtered by suction through Whatman GF/B glass fiber filters. The filters were placed in scintillation vials each containing 10.00 ml of a standard 2,5-diphenyloxazole/1,4-bis[2-(5-phenyloxazolyl)]benzene/toluene scintillation fluid containing 30% (vol/vol) Triton X-100 (Rohm and Haas) as solubilizer. The radioactivity in the vials was measured to less than 0.5% statistical error on a Beckman LSC 7500 liquid scintillation counter.

Binding was normalized per milligram of total protein, subtracting total observed binding from that of a control to compensate for nonspecific filter binding (21). Specific binding was defined as the difference in binding in the presence and the absence of a high concentration (10 μM) of (−)-isoproterenol.

**Calculations.** The results of the binding assays were expressed as Scatchard plots (22). Fluorescence intensities were available as peak heights directly from the spectra. Fluorescence depolarization and rotational relaxation times were calculated in the manner described by Weber (23). For depolarization

$$P = \frac{I_{\parallel} - I_{\perp}c}{I_{\parallel} + I_{\perp}c},$$

and the rotational relaxation time is given by

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(\frac{3\tau}{\rho}\right)$$

in which $P$ is the observed polarization, $I$ is intensity, $c$ is the monochromator polarization correction factor (20, 24), $P_0$ is the limiting polarization at infinite viscosity, $\tau$ is the singlet lifetime, in nanoseconds, of the fluorescent species, and $\rho$ is the desired rotational relaxation time. The equation for the rotational relaxation time applies to fluorophores in which the absorption and emission dipole moments are parallel, which is assumed to be the case for the propranolol–receptor complex.

Because propranolol is not principally associated with membrane lipids in the concentration range at which it is bound stereospecifically to the β-adrenergic receptor, it would not be valid to reduce these data to microviscosities (25). We prefer

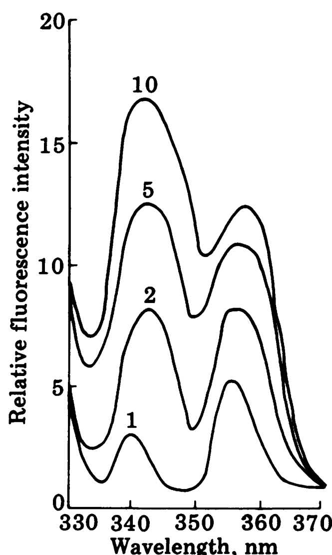


FIG. 1. Fluorescence emission spectra of propranolol in frog erythrocyte membrane fragment preparation. Concentrations of propranolol are denoted in pM above each spectrum. Excitation wavelength: 319 nm. This figure is similar to the emission spectrum in ref. 10.

to utilize the more phenomenological rotational relaxation time, which has been demonstrated (26) to be a satisfactory indicator of the membrane microenvironment.

**Fluorescence Lifetime Determination.** Singlet lifetimes were quantitated with the aid of a single-photon counting system (27). The actual lifetime of the propranolol was found to be well described as a single exponential, either by simple graphical analysis or by an iterative computer-based fitting routine. The lifetime was determined to be 13.3 ± 0.1 nsec.

## RESULTS

The unpolarized fluorescence emission spectrum of propranolol in the frog erythrocyte membrane fragment preparation is illustrated in Fig. 1. It is seen that the two signals at 356 and 340 nm—corresponding to specifically bound and free ligand, respectively—are very well resolved at the picomolar concentrations of propranolol utilized. As the total concentration of propranolol is increased, the relative magnitude of the ratio of the fraction bound to that which is free changes in accordance with the $K_d$ of the ligand from the receptor. This is better illustrated in Fig. 2, which is a Scatchard plot of the bound/free intensity ratio against the fluorescence signal of the bound ligand over a wide range of propranolol concentrations.

From the Scatchard plot, the $K_d$ is determined to be 6.11 ± 0.33 nM, and the $B_{max}$ is found to be 135.5 fmol/mg. This is in excellent agreement with our previous results from the radiobinding assay (10, 11) for the same preparation, which gave a $K_d$ of 4.72 ± 0.75 nM and a $B_{max}$ of 133.9 fmol/mg.

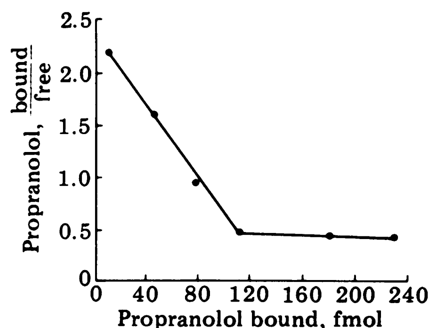


FIG. 2. Scatchard plot of propranolol binding to frog erythrocyte membrane fragment preparation. Ratios of bound/free and amounts bound were calculated from the corrected peak heights from the fluorescence emission spectrum. Each point represents the mean of three determinations. The figure is similar to the Scatchard plot of propranolol binding in ref. 10.

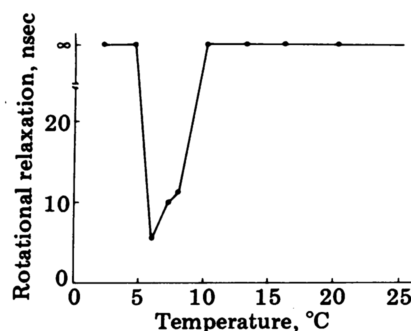


FIG. 3. Rotational relaxation for the propranolol–receptor complex versus the temperature of the fluorescence cuvette. Temperature was quantitated to a precision of 0.02°C.

Steady-state depolarization measurements of the bound propranolol band (356 nm) were obtained at each ligand concentration represented on the Scatchard plot. At a total propranolol concentration below 10 pM, the observed polarization at 20°C was the limiting polarization. As the concentration of the drug was increased, the polarization decreased. The degree of polarization $P$ was found to correlate directly with the relative intensity of the bound propranolol fluorescence; a linear least-squares analysis was performed and a correlation coefficient of 0.995 was obtained.

Depolarization measurements were obtained at temperatures from 1.0°C to 37.0°C. The fluorescence signal at 356 nm of bound ligand was used to calculate the rotational relaxation time of the propranolol–receptor complex as a function of temperature. These data are illustrated in Fig. 3. At temperatures from 2°C to 5°C the rotational relaxation time is observed to be infinite, as it is in the region from 10°C to 25°C. Interestingly, in the temperature range 6–10°C the rotational relaxation undergoes an apparent discontinuity, and becomes finite, its lowest value being 6.1 nsec at 6°C. The behavior in the range 6–10°C is fully reversible.

In order to determine if the observed release of constraint was an artifact of release of propranolol into solution in the temperature range 6–10°C, the binding of propranolol was examined as a function of temperature, with the aid of [$^3$H]propranolol as the ligand. These results are summarized in Fig. 4.

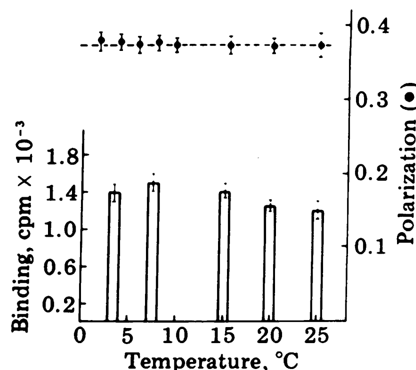


FIG. 4. (*Lower*) Total binding of [$^3$H]propranolol versus temperature in the frog erythrocyte membrane fragment preparation. The error bar at the top of each large bar represents the SEM for four duplicate determinations. The propranolol concentration in each tube was 1 nM. (*Upper*) Change in fluorescence polarization, $P$, with temperature for the probe 1,6-diphenylhexatriene incorporated into the frog erythrocyte membrane fragments at a final concentration of 0.1 $\mu$M.

It is seen that there is little variation in binding with temperature.

In order to evaluate the possible role of a membrane phase transition in the temperature-dependent behavior of the propranolol–receptor complex, the fluorescence depolarization of the nonspecific, lipophilic probe 1,6-diphenylhexatriene was examined as a function of temperature in the frog erythrocyte membrane fragment system. We have reported these results simply in terms of the observed polarization, owing to the difficulties in the interpretation of diphenylhexatriene data in terms of microviscosities (26). These results are illustrated in Fig. 4. No significant change occurs in the diphenylhexatriene polarization as a function of temperature in the region in which the release of constraint is observed.

In order to examine the possible importance of cytoskeletal factors in regulating the environment of the $\beta$-adrenergic receptor, a number of substances known to specifically disrupt microtubules or microfilaments were evaluated for their effects upon the rotational constraint of the propranolol–receptor complex. Colchicine at a concentration of 4 $\mu$g/ml caused a rapid drop in polarization, from a $P$ of 0.46 to 0.13, at 20°C. The effect of increasing concentrations of colchicine upon the polarization is illustrated in Fig. 5. It is seen that even very low concentrations of the drug have some effect upon the observed polarization, and that this effect is linear with dose up to nearly 4 $\mu$g/ml. Above this concentration a further increase in added drug appears to have little or no effect upon the polarization.

Other drugs that are known to act upon the cytoskeleton appear to behave in the present protocol in a manner qualitatively similar to that of colchicine. Vincristine and vinblastine both caused a rapid and irreversible drop in the polarization of the fluorescence signal from the propranolol–receptor complex when added at 4 $\mu$g/ml. Preliminary data indicate that the effect of all of these drugs is simply additive at concentrations below 4 $\mu$g/ml. Cytochalasin B at 10 $\mu$g/ml caused an initial drop in the polarization from its limiting value (0.46) to 0.27, followed by a slow return to the former value over a period of several minutes. Amphotericin B at 1 mg/ml was without effect upon the observed polarization at 20°C.

In attempting to validate the hypothesis that the drugs colchicine, vincristine, and vinblastine act solely upon the microtubular structure, the binding of the antagonist [$^3$H]dihydroalprenolol was examined at a colchicine concentration of 10 $\mu$g/ml, which is greater than that required for the maximal effect in the observed polarization. The result of the binding assay is illustrated in Fig. 6. It is seen that the $K_d$ is not influenced by the presence of colchicine. The only difference that can be ascribed to the added colchicine is the reduction of nonspecific binding to the preparation.

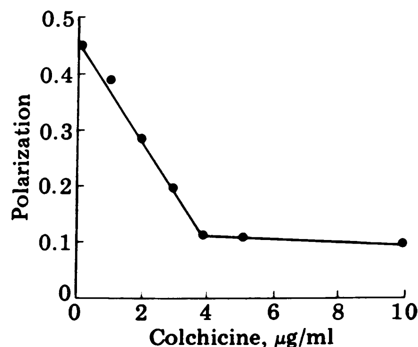


FIG. 5. Observed fluorescence polarization at 20°C of the propranolol–receptor complex as a function of added colchicine concentration. Total propranolol concentration was 5 pM.

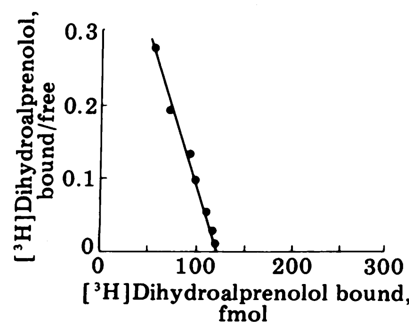


FIG. 6. Scatchard plot of the binding of [$^3$H]dihydroalprenolol to the frog erythrocyte membrane fragment preparation in the presence of colchicine at 10 $\mu$g/ml. Nonspecific binding is observed to be reduced under these conditions, whereas the $K_d$ and $B_{max}$ remain unperturbed from their values for this ligand measured in the absence of colchicine.

## DISCUSSION

We previously demonstrated that the drug propranolol can be applied as a fluorescence probe for the frog erythrocyte $\beta$-adrenergic receptor. This makes it possible to examine the microenvironment of the drug–receptor complex by the methodology of fluorescence depolarization.

At room temperature the receptor–ligand complex appears to be rotationally immobilized within the singlet lifetime of the probe. This is in itself not surprising, because large-scale lateral diffusion would not be expected to be observed on a nanosecond time scale. Any rotational motion that would be observed might be expected to result from side-chain motion in the complex as an accompaniment to lateral diffusion (28).

However, the influence of temperature upon the observed polarization of the propranolol–receptor complex is indeed unexpected. An initial interpretation is that the observed behavior is the result of either of two possible processes. First, the change in temperature might well induce a global order–disorder transition, which would presumably result in altered mobility of the propranolol–receptor complex. This change could be reflected in the observed loss of constraint. Alternatively, the change in temperature could result in release of propranolol from the receptor. This free propranolol could then cause an apparent reduced polarization.

We have evaluated the possible participation of both of these processes, and have found that neither accounts for the temperature-dependent decrease in the polarization. As Fig. 4 illustrates, there is no significant change in the polarization of diphenylhexatriene in the temperature range in question. This rules out the possibility of a membrane fluidity transition that is macroscopically observable. The fact that the observed antagonist binding varies little with temperature suggests that the second hypothesis has little validity.

Two alternative explanations persist to account for the present data. First, the lipid environment in which the $\beta$-adrenergic receptor resides may be partitioned in such a manner that the diphenylhexatriene polarization does not serve as an accurate reporter of its microenvironment. Either chemical differences or physical heterogeneity of the boundary lipids adjacent to the receptor could account for such an isolated microenvironment. Alternatively, cytoskeletal involvement with the $\beta$-adrenergic receptor could account for the observed temperature dependence. Microtubular depolymerization might be expected to occur very sharply in the temperature regime in which the polarization decrease is observed (29). Also, the reversibility of the transition would be fully consistent with this explanation.

The possibility of cytoskeletal involvement with the $\beta$-adrenergic receptor has been raised previously (30), but there is to the best of our knowledge no direct experimental evidence to date supporting or negating this notion. Only in the rather different system of lectin receptors on lymphocyte surfaces has involvement with the cytoskeletal system been demonstrated (31).

Our results with colchicine, vincristine, vinblastine, and cytochalasin B demonstrate that the propranolol–receptor complex is cytoskeletally linked. No alternative hypotheses could account for the actions of these drugs, at concentrations consistent with their specific action upon the microtubular system. Furthermore, the absence of an influence by colchicine on either the $B_{max}$ or the $K_d$ of the antagonist [$^3$H]dihydroalprenolol indicates that microtubular disruption does not alter the affinity of the receptor for ligand. This result would be expected if the function of the cytoskeleton was to maintain a fixed spatial relationship between the receptor and its effector. A number of models have been proposed to account for an interaction of the $\beta$-adrenergic receptor with its associated adenylate cyclase and nucleotide regulatory protein (30, 32). We can neither positively support nor invalidate existing models. The present data suggest an alternative hypothesis in which agonists cause the receptor that is normally cytoskeletally linked to become unbound from the cytoskeleton. The *release* of the receptor from an inactive complex that is cytoskeletally linked results in activation of the adenylate cyclase. The kinetics of such a model would fit those of the collisional coupling hypothesis of Tolkovsky and Levitzki (32). By postulating that the removal of the receptor subunit activates the cyclase, the effects of acyl chain ordering (33, 34) or altered membrane viscosity (35) on the activation of the adenylate cyclase can be readily understood in terms of the altered ability of the receptor to move away from the cytoskeletally linked complex.

Our results provide convincing evidence that the $\beta$-adrenergic receptor is linked to the microtubules of the cellular cytoskeleton in the frog erythrocyte. No previously published mechanism for the interaction of the $\beta$-adrenergic receptor considers the involvement of the cell cytoskeleton, and in fact it appears to be implicitly assumed that the receptor freely floats in the membrane. It is apparent from the present data that this notion is not correct, and new models are necessary to explain the cytoskeletal involvement in the $\beta$-adrenergic receptor system.

**Note Added in Proof.** We have observed in some samples of propranolol an impurity with an excitation maximum at 290 nm and an emission maximum at 340 nm which is removed by recrystallization from 1-propanol. The recrystallized material behaves as reported above.

We thank Profs. N. E. Geacintov and H. C. Brenner for critical comments on the manuscript, and assistance with instrumentation. Technical assistance was provided by Mr. W. Holden and Mr. L. Garretson. This work was supported by National Institutes of Health Grants EY 01340, EY 07009, and GM 25002 (to J.A.Z.) and DA 0009 (to R.B.M.).

1. Zadunaisky, J. A., Schaeffer, B. & Cherksey, B. D. (1980) *Ann. N.Y. Acad. Sci.* **341**, 233–245.
2. Klainer, L. M., Chi, Y. M., Freidberg, S. L., Rall, T. W. & Sutherland, E. W. (1962) *J. Biol. Chem.* **237**, 1239–1243.
3. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1971) in *Cyclic AMP* (Academic, New York), pp. 150–152.
4. Lefkowitz, R. J., Limbird, L. L., Mukherjee, C. & Caron, M. (1976) *Biochim. Biophys. Acta* **457**, 1–39.
5. Vatner, D. & Lefkowitz, R. J. (1974) *Mol. Pharmacol.* **10**, 450–456.
6. Atlas, D., Steer, M. L. & Levitzki, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4246–4248.
7. Weber, G. & Farris, F. J. (1979) *Biochemistry* **18**, 3075–3078.
8. Pohev, L. T. (1967) *J. Pharmacol. Exp. Ther.* **155**, 91–100.
9. Shand, D. J., Nickolls, E. M. & Oates, J. A. (1970) *Clin. Pharmacol. Ther.* **11**, 112–120.
11. Cherksey, B. D., Zadunaisky, J. A. & Murphy, R. B. (1980) *Proceedings of the Conference on Forms of Microtubule Organization in Cells*, eds. Albrecht-Buehler, G. & Anderson, R. G. W. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 11 (abstr.).
(Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 11 (abstr.).
12. Lefkowitz, R. J., Mullikin, D. & Caron, M. G. (1976) *J. Biol. Chem.* **251**, 4686–4692.
13. Belford, G. C., Belford, R. C. & Weber, G. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1392–1395.
14. Lakowicz, J. R., Prendergast, F. G. & Hogen, D. (1979) *Biochemistry* **18**, 508–519.
15. Gilmore, R., Cohn, N. & Glaser, M. (1979) *Biochemistry* **18**, 1043–1049.
16. Golan, D. E. & Veatch, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2537–2541.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
18. Christmann, D. R., Crouch, J. R., Holland, J. F. & Timnick, A. (1980) *Anal. Chem.* **52**, 291–294.
19. Weber, G. (1972) *Annu. Rev. Biophys. Bioeng.* **1**, 553–587.
20. Vanderkooi, J., Fischkoff, S., Chance, B. C. & Cooper, R. A. (1974) *Biochemistry* **13**, 1589–1595.
21. Seeman, P., Tedesco, J. L., Lee, T., Chau-Wong, M., Muller, P. Bowles, J., Whitaker, P. M., McManus, C., Tittler, M., Weinreich, P., Friend, W. C. & Brown, G. M. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 130–136.
22. Tanford, C. (1960) *Physical Chemistry of Macromolecules* (Academic, New York), pp. 526–548.
23. Weber, G. (1952) *Biochem. J.* **51**, 145–167.
24. Weber, G. (1973) in *Fluorescence Techniques in Cell Biology*, eds. Thaer, A. A. & Sernetz, M. (Springer, Berlin), pp. 133–158.
25. Yguerabide, J. (1972) *Methods Enzymol.* **26**, 498–578.
26. Dale, R. E., Chen, L. A. & Prand, L. (1977) *J. Biol. Chem.* **252**, 7500–7510.
27. Geacintov, N. E., Prusik, T. & Khosorofian, J. F. (1976) *J. Am. Chem. Soc.* **98**, 6444–6452.
28. Cherry, R. J. (1980) *Biochim. Biophys. Acta* **559**, 290–327.
29. Nicholson, G. (1976) *Biochim. Biophys. Acta* **457**, 40–85.
30. Rodbell, M. (1980) *Nature (London)* **284**, 17–22.
31. Schlessinger, J., Elson, E. L., Webb, W. W., Yahara, I., Rutishauser, U. & Edelman, G. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1110–1114.
32. Tolkovsky, A. M. & Levitzki, A. (1978) *Biochemistry* **17**, 3795–3810.
33. Sinesky, M., Minneman, K. P. & Molinoff, P. B. (1979) *J. Biol. Chem.* **254**, 9135–9141.
34. Sinesky, M., Pinkerton, F., Sutherland, E. & Simon, F. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4893–4897.
35. Bakardjieva, A., Galla, H. J. & Helmreich, E. J. M. (1979) *Biochemistry* **18**, 3016–3023.