Characterization of an Altered Membrane Form of the β-Adrenergic Receptor Produced during Agonist-induced Desensitization*

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Incubation of 1321N1 human astrocytoma cells with 1 μM isoproterenol rapidly results in the conversion of a portion of the β-adrenergic receptors to a membrane form that can be separated from markers for the plasma membrane by sucrose density gradient or differential centrifugation. This "light peak" form of the receptor reaches a maximal level within 10 min of incubation of cells with catecholamine. Two types of experiments suggest that the early phase of catecholamine-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase can be separated into at least two reactions. First, the agonist-induced loss of catecholamine-stimulated adenylate cyclase activity precedes the appearance of β-adrenergic receptors in the light peak fraction by 1–2 min. Second, pretreatment of cells with concanavalin A prior to induction of desensitization blocks the formation of the light peak form of β-adrenergic receptors without blocking the "uncoupling" reaction as measured by catecholamine-stimulated adenylate cyclase activity. Specificity for the reaction that converts β-adrenergic receptors to the light peak form is indicated by the lack of a catecholamine-induced alteration in the sucrose density gradient distribution of muscarinic cholinergic receptors, adenylate cyclase or the guanine nucleotide-binding proteins, N1, and N2. The light peak of β-adrenergic receptors migrates at a density similar to that of at least a portion of the activity of galactosyltransferase, a marker for Golgi. Enzyme marker activities for lysosomes and endoplasmic reticulum are not associated with this population of β-adrenergic receptors. Taken together, these and other data suggest that incubation of 1321N1 cells with isoproterenol results in a rapid uncoupling of β-adrenergic receptors from adenylate cyclase which is followed by a change in the membrane form of the receptor. This latter step most likely represents internalization of receptors into a vesicular form which may then serve as the precursor state from which receptors are eventually lost from the cell.

cells has been shown to involve at least two reactions (1–3). First, cells exposed to catecholamines experience a rapid (1/2 ~ 2 min) functional uncoupling of receptors from adenylate cyclase which is expressed as a 50% decrease in the responsiveness of the enzyme to agonists and a loss of capacity of agonists to form a high affinity binding complex (1, 3). These alterations are rapidly (1/2 ~ 7 min) and completely reversible upon removal of catecholamine from the medium (3). A second reaction that occurs later upon desensitization of the β-adrenergic receptor antagonist binding occurs after a lag of 30–60 min (2–4); the formation and rate of recovery of lost receptors is much slower than the changes occurring during short-term exposure of cells to agonist (3, 4). Similar phenomena have been shown to occur in a variety of other cell types during exposure to catecholamines (5–9).

We have reported recently that short-term incubation of 1321N1 cells with isoproterenol also results in the accumulation of a subpopulation of receptors that exhibits altered sedimentation properties on sucrose density gradients (10). The time course of appearance of this population of receptors roughly coincided with the agonist-induced uncoupling reaction. Furthermore, the agonist binding properties of this receptor population were equivalent to that of "uncoupled" β-adrenergic receptors. Exposure of frog erythrocytes to catecholamines results in the appearance of a similar subpopulation of β-adrenergic receptors in supernatant fractions of lysates (11–13). We describe in the present report results from experiments that further characterize the agonist-induced modification of the membrane form of the β-adrenergic receptor in 1321N1 astrocytoma cells.

EXPERIMENTAL PROCEDURES

1 "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 8560 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1278, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations are: ConA, concanavalin A; Heps, 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid; 125I-PIN, (125I)iodo-1-pindolol; [3H]QNB, (-)-[3H]quinuclidinyl benzilate; IAP, islet-activating protein; N1, the stimulatory guanine nucleotide regulatory component of adenylate cyclase; N2, the inhibitory guanine nucleotide regulatory component of adenylate cyclase; C, the catalytic component of adenylate cyclase; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; GTPγS, guanosine 5'-3(thiotriphosphate).
RESULTS

As we have previously reported (10), short-term (15 min) incubation of 1321N1 cells with isoproterenol results in an alteration in the distribution of β-adrenergic receptors on sucrose density gradients (Fig. 1). Rather than migrating primarily as a single peak at a density equivalent to that of other markers of the plasma membrane, a large portion of the receptors migrate as a "light peak" at sucrose densities of 30% or less. The properties of these light peak receptors have been compared to those of receptors from the plasma membrane fraction and from cell lysates. Saturation binding isotherms for 125I-IPN were generated with receptors from the light peak and from the plasma membrane fraction derived from both control and desensitized cultures (Fig. 2). The affinity (12-15 PM) of 125I-IPN in each receptor-containing fraction was similar to that (14 PM) determined in cell lysates (data not shown). The kinetics of 125I-IPN binding also were examined for each receptor fraction. The rate constants for association (k Eagle) of 125I-IPN were 8.4 X 10^(-5), 8.3 X 10^(-4), and 11.7 X 10^(-4) M^-1 min^-1 for the control lysate, desensitized light peak, and desensitized plasma membrane fraction, respectively. The rate constants for dissociation (k_dissociation) were 0.015, 0.031, and 0.021 min^-1 for the same three receptor-containing fractions. The kinetically determined dissociation constants (k_dissociation/k_association) were in good agreement with the K_d values determined by equilibrium assay.
of control.

Loss during short-term exposure of 1321N1 cells to 1 p~ isoproterenol for 20 min with catecholamine are usually 90-100% of control, in

has occurred. Although receptor levels in lysates from cells pretreated

binding of ²²¹-PIN was measured as a function of ligand concentra-

ation (3-300 PM) at a protein concentration of approximately 15 pg/

assay.

incubated in the absence or presence of 1 p~ isoproterenol

in a  Beckman 42.1 Ti rotor. The pellets were diluted with 145 mM

in the light peak and plasma membrane fractions. Cells were

then prepared and centrifuged on a continuous 30-60% sucrose

gravities each were pooled and diluted with 0.25 mg/ml of ConA. Dishes were then placed on ice for 20 min, followed by

hypotonic lysis as described earlier. Aliquots of the lysates were saved for determination of isoproterenol (10 μM)-stimulated adenylate cyclase activity (O), and the remaining lysate was layered on a 30-60% sucrose gradient and centrifuged as described under “Experimental Procedures.” The specific binding of ²²¹-PIN (O) in the light peak fractions was summed and plotted as a per cent of maximal activity. Isoproterenol-stimulated adenylate cyclase activity is plotted as the per cent of activity in control lysates. The data is the mean ± S.E. of four experiments.

Although the total number of receptors is only minimally reduced during short-term (20 min) incubation of 1321N1 cells with isoproterenol (1-3), as we have previously described in detail, extended incubation results in a marked reduction in measurable receptors (2-4). Thus, the temporal relationship of receptors appearing in the light peak to those in the plasma membrane fraction was examined. Maximal conversion of receptors to the light peak form occurred within 10 min during incubation of cells with 1.0 μM isoproterenol. Once

altered form of receptors to the light peak form occurred, receptors were lost from this and the plasma membrane fraction with similar time courses (Fig. 1). Thus, a precursor-product relationship could not be established directly for the light peak form of receptors in generating receptors that are lost from the cell.

The appearance of receptors in the light peak fraction occurs during a time when a major decrement in catechol-

amine-stimulated adenylate cyclase activity occurs. Thus, attempts were made to define in detail the temporal relationship between the change in the membrane form of β-adrenergic receptors and the uncoupling of adenylate cyclase as measured by a loss of isoproterenol-stimulated enzyme activity. As we have previously reported (3), catecholamine-stimulated adenylate cyclase activity is rapidly lost (t½ = 2.5 min) during incubation of cells with isoproterenol to a level that is approximately 50% of control (Fig. 3). Although catecholamine-stimulated activity was reduced by 20-25% during the first 1-2 min of incubation with isoproterenol (Fig. 3 and Ref. 3), no significant change (p > 0.05) occurred in the number of receptors appearing in the light peak (Fig. 3). After this lag of 1-2 min, a conversion of β-adrenergic receptors to the light peak form occurred, with maximal levels of receptor appearing in this fraction within 10 min (t½ = 4.5 min).

Such kinetic experiments (Fig. 3) indicated that, at least at very short times of incubation, the uncoupling reaction as measured by isoproterenol-stimulated adenylate cyclase activity could be distinguished from the change in the membrane form of receptors. This distinction was pursued further by examining the effects of various chemicals on these two

Fig. 2. Scatchard analyses of ²²¹-PIN binding to receptors in the light peak and plasma membrane fractions. Cells were incubated in the absence or presence of 1 μM isoproterenol (ISO) for 15 min. Lysates from nine 150-mm dishes for each condition were then prepared and centrifuged on a continuous 30-60% sucrose gradient as described under “Experimental Procedures.” The light peak (O) and plasma membrane (Δ) fractions of control and desen-

sitized gradients each were pooled and diluted with 1 μM Tris (pH 7.4 at 4 °C) and pelleted by centrifugation for 60 min at 170,000 × g in a Beckman 42.1 Ti rotor. The pellets were diluted with 145 mM NaCl, 20 mM Tris (pH 7.5 at 25 °C), and 5 mM MgCl₂, and specific binding of ²²¹-PIN was measured as a function of ligand concentration (3-300 pM) at a protein concentration of approximately 15 μg/assay.

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processes. Preincubation of cells with 0.25 mg/ml of ConA in buffered Eagle's medium at 37 °C with or without ConA and pretreated cells was the same as in control cells (Fig. 4). Thus, with catecholamine should not be confused with the routine use of completely blocked the agonist-induced conversion of membrane form of β-adrenergic receptors. The effect of ConA on adenylate cyclase activities were measured in pooled fractions preincubated for 45 min at 37 °C with ConA and gradients generated as described under "Experimental Procedures." The data are representative of six experiments. Right, isoproterenol-stimulated adenylate cyclase activity was determined in lysates from cells treated with ConA and/or isoproterenol (ISO) as described for the left panel of this figure. The data are expressed as the percent of the value for isoproterenol-stimulated activity divided by basal activity for control cells. The data are the mean ± S.E. for four experiments.

Although there was a 50% decrease in isoproterenol-stimulated adenylate cyclase activity in ConA-treated cells, the cultures were washed three times with Hepes-buffered Eagle's medium with another 15 min. After incubation with isoproterenol and ConA, the cultures were washed three times with Hepes-buffered Eagle's medium at 37 °C with or without ConA and incubated for an additional 45 min at 37 °C. There was no effect of ConA on the capacity of cells to recover catecholamine-induced desensitization also was examined (data not shown). Cells were either desensitized for 30 min or desensitized for 15 min then treated with 0.25 mg/ml of ConA in the continued presence of isoproterenol for another 15 min. After incubation with isoproterenol and ConA, the cultures were washed three times with Hepes-buffered Eagle's medium at 37 °C with or without ConA and incubated for an additional 45 min at 37 °C. There was no effect of ConA on the density of β-adrenergic receptors was determined from Scatchard analyses of 125I-PIN binding to the fractions.  

The activity of N, was determined in extracts of the membrane fractions prepared as described under "Experimental Procedures." Extracts were diluted with a solution containing 1 mM sodium/EDTA, 1 mM dithiothreitol, 0.1% Lubrol-12A, 10 mM MgCl2, and 10 mM GTP-S, and incubated for 60 min at 30 °C to activate Ncyc membranes were reconstituted with three dilutions of each extract, and the specific activity of N, was determined from these linear reconstitutions of adenylate cyclase with added extract. The activities are expressed per mg of the original membrane protein.

The activity of the catalytic component of adenylate cyclase was determined by stimulation with a saturating concentration of purified rabbit liver N, which had been activated with GTP-S. Resultant adenylate cyclase activity was measured as described under "Experimental Procedures."  

A concentration-effect curve for the effects of Lubrol-PX on adenylate cyclase activity was generated for the light peak and plasma membrane fraction of gradients. An approximately 8-fold increase in basal or NaF-stimulated adenylate cyclase activity occurred in the presence of detergent in the light peak fractions. The optimal concentration for this effect was 0.05% Lubrol-PX. Only minor (~2-fold increase) effects of Lubrol-PX on enzyme activity were observed in assays with the plasma membrane fraction.
Fig. 5. Adenylate cyclase activity in sucrose density gradient fractions generated from control and desensitized cells. Peak regions from two control (solid bars) and two desensitized (open bars) gradients were prepared as described in the legend to Fig. 2. Basal, isoproterenol (ISO), NaF, and NaF + Lubrol-PX (0.03%)-stimulated adenylate cyclase activities were measured in both the light peak and the plasma membrane (heavy peak) preparation. Samples were treated in the absence or presence of detergent for 20 min on ice immediately prior to assay. The specific binding of $[^{125}]$I-PIN was determined as described under "Experimental Procedures. $\beta$-AR, $\beta$-adrenergic receptors.

We also have examined the distribution of the components of adenylate cyclase in the light peak and plasma membrane fractions from both control and desensitized cells. Table I presents the results of the quantitative activities assayed for N, and C. These results, in contrast to those for the $\beta$-adrenergic receptor, show that the specific activities of N, and C are identical in membrane fractions prepared from control or desensitized cells. In both control and desensitized cells, the specific activities of N, and C are 5-10-fold lower in the light peak fraction than in the plasma membrane fraction.

We also attempted to determine if desensitization involves a modification of N, or N, by examining the cholera toxin and IAP labeling patterns of membrane fractions prepared from control or desensitized cells. Fig. 6 presents an autoradiogram from such an experiment. All four membrane fractions display the same pattern of labeling with cholera toxin or IAP. Cholera toxin labels predominantly 52,000-, 45,000-, and 42,000-dalton substrates from these membranes, and the intensity of labeling correlates well with the N, activities measured in reconstitution assays with cyc- membranes. In other preparations of 1321N1 cell membranes, the 42,000-dalton cholera toxin substrate was absent, suggesting that it may be a proteolytic product of the 52,000- or 45,000-dalton substrate. The predominant IAP substrate in all four membrane fractions was a 41,000-dalton peptide, as seen by Katada and Ui (36, 37) for C6 glioma cells. As with the cholera toxin substrates, the IAP substrate does not appear to be enriched in
FIG. 7. Comparison of sucrose density gradient profiles of β-adrenergic and muscarinic cholinergic receptors. Sucrose density gradients were generated from lysates of control (○) and desensitized (△) 1321N1 cells (1 μM isoproterenol; 20 min) as described under "Experimental Procedures." The distribution of β-adrenergic receptors (left) was determined with [125I]-PIN, and the distribution of muscarinic receptors (right) was determined with [3H]-QNB. The data for [3H]-QNB are representative of three experiments.

FIG. 8. Gradient distribution of marker enzymes and β-adrenergic receptors. A, the distribution of β-adrenergic receptors from control (○) versus desensitized (△) cells (15 min; 1 μM isoproterenol) was compared on a 15-30% sucrose continuous gradient topped with a step of 5% sucrose (see "Experimental Procedures"). B, cells were preincubated for 15 min with 1 μM isoproterenol, and the distribution of acid phosphatase (○), galactosyltransferase (△), and β-adrenergic receptors (●) was assessed on the gradient described above. The per cent of total activity recovered in the pellet fractions was 30-45% for galactosyltransferase and 20-30% for acid phosphatase.

The desensitized light peak fraction as compared with control cells.

Further evidence for the specificity of the isoproterenol-induced alteration in the gradient distribution of β-adrenergic receptors was provided by monitoring the fate of another membrane-bound receptor, the muscarinic cholinergic receptor. Under conditions where 40-50% of the β-adrenergic receptors were converted to the form appearing in the light peak, no change occurred in the density gradient profile of muscarinic receptors identified (38) by [3H]-QNB binding (Fig. 7).

One interpretation of the data indicating a change in the membrane form of β-adrenergic receptors during incubation of 1321N1 cells with isoproterenol is that an agonist-induced internalization of the β-adrenergic receptor has occurred. Thus, the extent to which β-adrenergic receptors in the altered membrane form co-migrated with markers for subcellular organelles was examined. The distribution on shallow sucrose density gradients of marker enzymes for Golgi, endoplasmic reticulum, and lysosomes was compared to that of β-adrenergic receptors appearing in the light peak. Lysates from cells previously incubated for 20 min with 1 μM isoproterenol were resolved on a 15-30% sucrose density gradient. No apparent association of [125I]-PIN binding activity with lysosomal enzyme marker activity (acid phosphatase) occurred (Fig. 8B). The marker for endoplasmic reticulum (glucose 6-phosphatase) exhibited a distribution similar to that of acid phosphatase on these shallow gradients and thus does not appear to be associated with the light peak of β-receptors (data not shown). While the majority of galactosyltransferase activity migrated at a different density, a small amount of this activity co-migrated with the light peak of β-adrenergic receptors (Fig. 8B).

One possible concern in interpreting these results is that β-adrenergic receptors in an altered membrane form might exhibit different densities depending upon the point in a process of internalization and cellular processing at which they were examined. However, the peak of [125I]-PIN binding activity that occurred at 18-22% sucrose on the gradient illustrated in Fig. 8 migrated at the same density irrespective of the time (5-60 min) of incubation with isoproterenol (data not shown). As illustrated in Fig. 8A, the β-adrenergic receptors from control cells did not migrate as a distinct peak, but rather were broadly distributed throughout the 15-30% sucrose gradient. Adenylate cyclase activity also did not migrate as a distinct peak in these gradients (data not shown).

The results illustrated above were all obtained utilizing sucrose density gradients to resolve the altered membrane form of the β-adrenergic receptor from receptors associated with the plasma membrane. On the basis of this difference in density, the two receptor-containing fractions also can be resolved by differential centrifugation. As is illustrated in Fig.
9A, the majority of $\beta$-adrenergic receptors in control lysates were pelleted by a 10-min centrifugation at 15,000 $\times$ g. This g-force ranged from 10,000 to 15,000 $\times$ g in four experiments. In contrast, while greater than 60% of the $\beta$-adrenergic receptors in lysates from cells previously incubated (15 min) with isoproterenol were pelleted at the same low centrifugation forces, the remaining receptors were only pelleted at much greater forces (Fig. 9A). The force (30,000 to greater than 50,000 $\times$ g) necessary for sedimenting in a 10-min centrifugation all of the $\beta$-adrenergic receptors in lysates from desensitized cells was variable. This result is not surprising due to variations in the amount of tissue/ml in these experiments and inaccuracies inherent in this type of experiment with a preparative centrifuge. The data suggest that the altered membrane form of the receptors in lysates from desensitized cells can be resolved from the plasma membrane form simply by differential centrifugation. This point is more directly assessed in Fig. 9B. Receptors migrating in the light peak and plasma membrane fractions from desensitized cells were diluted to equivalent concentrations (~4%) of sucrose and subjected to differential centrifugation (Fig. 9B). Whereas receptors from the plasma membrane fraction were sedimented at between 20,000 and 50,000 $\times$ g, the majority of receptors in the light peak fraction were not sedimented by a 10-min centrifugation at 50,000 $\times$ g.

**DISCUSSION**

The results of the present study provide evidence that the early phase of catecholamine-induced desensitization of adenylate cyclase can be separated into at least two different reactions. On a kinetic basis, "uncoupling" or loss of hormonal responsiveness occurs earlier than does formation of the light peak fraction of $\beta$-adrenergic receptors. This conclusion is supported by the observation that treatment of cells with ConA prevents formation of the light peak receptor fraction under conditions where neither the uncoupling reaction nor the recovery reaction are measurably altered. Thus, the following set of reactions can be proposed as a working hypothesis for the mechanism of receptor-specific, agonist-induced desensitization in 1321N1 cells:

$$\begin{align*}
\beta \text{AR}_N \rightarrow +A & \rightarrow \beta \text{AR}_i \\
\beta \text{AR}_i \rightarrow +A & \rightarrow \beta \text{AR}_p \\
\beta \text{AR}_p \rightarrow +A & \rightarrow \beta \text{AR}_i
\end{align*}$$

where $\beta \text{AR}_N$, $\beta \text{AR}_i$, $\beta \text{AR}_p$, and $\beta \text{AR}_i$ are the native, uncoupled, light peak, and lost (1-4, 10) forms of the receptor, respectively, and A is a receptor agonist.

The observation that Reactions 1 and 2 are rapidly reversible (3) and that Reaction 3 is relatively slow in the forward direction and very slow in the reverse direction (3, 4) provide an explanation for the similar kinetics of loss of $\beta \text{AR}_N$ and $\beta \text{AR}_p$ during continued exposure to isoproterenol (Fig. 1). In other words, conversion of a few molecules of $\beta \text{AR}_p$ to $\beta \text{AR}_i$, would result in a rapid adjustment of the steady state levels of $\beta \text{AR}_N$, $\beta \text{AR}_i$, and $\beta \text{AR}_p$; the consequence is the near parallel rates of loss of $\beta \text{AR}_N$ and $\beta \text{AR}_p$ over an 11-h exposure to isoproterenol.

The results of the present study also provide further evidence for the specificity of the agonist-induced, receptor-specific desensitization process. Isoproterenol causes no movement of muscarinic cholinergic receptors to the light fractions of sucrose gradients. In addition, adenylate cyclase activity is not observed to increase in the light peak after exposure to isoproterenol nor are its components when they are assayed directly.

The presence of significant amounts of NaF-stimulated enzyme activity in the light peak region of both control and desensitized gradients is revealed by treatment of the samples with 0.03% Lubrol-PX. One interpretation of such results is that the light peak region is contaminated with right side-out vesicle fragments of the plasma membrane. If such were the case, these vesicles would not have measurable adenylate cyclase activity since ATP would have limited access to the active site of the enzyme within the vesicle. Lubrol would be expected to permeabilize the membrane to ATP. The fact that Lubrol caused a large increase (8-fold) in activity in the light peak but only a minor increase (2-fold) in enzyme activity in the heavy peak region (which contains primarily open sheets of plasma membrane) is consistent with this interpretation.

The experimental results shown in Table I and Fig. 6 indicate that no detectable transfer of N, or N, into the light peak fractions occurs upon exposure of cells to isoproterenol. However, the results cannot exclude the possibility that a complex with a 1:1 stoichiometric ratio of N, to $\beta$-receptor is shifted to these fractions. Based on our calculations, the N, content of the light peak fractions of control samples (Table I) is about 580 fmol. A bout 70 fmol of $\beta$-receptor were

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$^6$Northup et al. (48) have determined that the specific activity of
transferred to the light peak fractions during desensitization. In view of the potential variation in estimates of \( N_e \), it seems unlikely that we could distinguish values for \( N_e \) of 580 and 650 fmol before and after desensitization. In fact, in the experiment shown (Table 1), less \( N_e \) was found in the light peak fractions from desensitized cells. This probably is primarily a reflection of a difference in protein content since the percentage of total \( N_e \) in the light fractions was the same in control and desensitized preparations, and, as shown, the specific activity of \( N_e \) was slightly higher in the desensitized sample. Stadel et al. (13) have carried out similar experiments using frog erythrocytes. They concluded that \( N_e \) was not sequestered along with the \( \beta \)-receptor into a light vesicle during desensitization. The conclusion was based on a reconstitution assay (22) for \( N_e \), similar to that used in our studies. Their results indicate that, after desensitization, the vesicle fraction contains about 11% of the \( N_e \) contained in the plasma membrane fraction of control cells. The \( N_e \) content of the vesicle fraction of control cells was not reported. Although Stadel et al. (13) did not report sufficient details to allow us to make the calculation described above, it is clear that the proportion of \( N_e \) in light and heavy peaks after desensitization in either frog erythrocytes or 1321N1 cells is low (11–14%) and that \( N_e \) is not transferred to the light vesicle fractions in proportion to the percent movement of \( \beta \)-receptors. However, it is probable that the results from frog erythrocytes (13) need to be accepted with the same caveats as indicated for the results with 1321N1 cells.

The studies of marker enzyme distribution failed to demonstrate clear patterns of co-migration of the \( \beta AR \) with the Golgi apparatus, endoplasmic reticulum, or lysosomes. However, these results cannot unequivocally exclude an association of the agonist-modified receptors with any of these organelles.

It is of interest to understand the nature of the \( \beta \)-receptors that appear in the light peak fraction of control gradients. Based on an estimate of plasma membrane contamination of the light peak, it can be predicted that no more than 10% of the total measurable \( \beta \)-receptors should appear in this fraction of gradients from control cells. In fact, we routinely find 8–12% of the total \( \beta \)-receptors in the light peak. This leads to the somewhat anomalous set of observations that on the one hand all of the \( \beta \)-receptors in the light peak from control gradients can be accounted for by plasma membrane contamination, i.e., native \( \beta \)-receptors, but on the other hand we previously observed that these receptors exhibited an uncoupled behavior in agonist competition binding studies (10). The lack of high affinity binding of agonist in the absence of GTP is a property we and others have attributed to the uncoupled state of the receptor during short-term desensitization (1, 8, 39). One possible explanation for this anomaly is that the contaminating plasma membrane fragments containing the receptor do not contain \( N_e \), which is required for the expression of high affinity binding of agonist. Ross et al. (15) have calculated that if the \( \beta \)-adrenergic receptor and adenylyl cyclase components of \( S_49 \) lymphoma cells were randomly and independently distributed in the plasma membrane, then they both should not be found together in membrane frag-

homogeneous preparations of rat liver \( N_e \) range between 7 and 17 \( \mu \)mol/min/mg of \( N_e \) when assayed according to standard procedures (22). Assuming a similar turnover number and molecular weight for the \( N_e \) of 1321N1 cells, we have used the data in Table 1 to calculate the amount of \( N_e \) in light and heavy peak fractions from sucrose gradients. These values (based on a specific activity of 12 \( \mu \)mol/min/mg of \( N_e \)) are: control light peak, 582 pmol; control heavy peak, 3475 pmol; desensitized light peak, 430 pmol; desensitized heavy peak, 2580 pmol.

ments of the size observed in their studies. Also, Sahyoun et al. (40) observed that fragmentation by sonication of membranes containing catecholamine-responsive adenylate cyclase resulted in a fraction of smaller membrane fragments which contained both \( \beta \)-adrenergic receptors and adenylyl cyclase activity, but hormone responsiveness was lost. Alternatively, receptors involved in transit to or from the plasma membrane could contribute to this fraction that no longer co-migrates with markers for the plasma membrane. Indeed, in contrast to desensitized cells, the receptors in the light fractions from control gradients exist as a rather broad band with little evidence of a distinct peak of activity (Fig. 8A), suggesting that a heterogeneous set of receptor-containing structures contributes to this activity. Thus, the lack of high affinity binding of agonist and guanine nucleotide effects on binding in the light peak fraction from control cells could be due to any of several factors that would result in a lack of normal interaction of \( \beta \)-adrenergic receptors and \( N_e \).

We and most other workers in this field have routinely utilized a 10-20-min centrifugation at 20,000–40,000 \( \times g \) to prepare a membrane fraction for the determination of receptor levels during catecholamine-induced desensitization. In retrospect, this is a less than optimal practice that has a number of ramifications in relation to the data presented in Fig. 9. Thus, receptors discussed as lost (degraded?) due to their failure to be detected in a sedimented membrane fraction may in fact be present in a form such as that in the light peak that does not sediment under the given conditions for centrifugation. These receptors might indeed be "lost" in a functional sense but could rapidly retain their functional association with adenylyl cyclase upon removal of catecholamine from the medium and thus would be distinguishable from receptors that were degraded or modified in a long-lasting way. Variabilities in time and other conditions of centrifugation could lead to variabilities in the extent to which receptors were recovered in the sedimentable fraction. As pointed out earlier, we have observed variabilities in the loss of receptors at early time points of desensitization. Stadel et al. (13) have reported recently that essentially all of the \( \beta \)-adrenergic receptors that are lost from a plasma membrane fraction during incubation of frog erythrocytes with isoproterenol can be recovered in a sedimentable form by centrifugation at 158,000 \( \times g \).

The catecholamine-induced conversion of \( \beta \)-adrenergic recep-
tors to another membrane form that exhibits properties similar to those observed with 1321N1 cells has been reported for frog erythrocytes (11–13) and C6 rat glioma cells (41). Since the kinetics of occurrence of catecholamine-specific desensitization are remarkably similar in these three systems, as well as in a number of other cell types (5, 7–9), it is likely that the phenomenon studied in the present manuscript represents a generally occurring response of the \( \beta \)-adrenergic receptor system to catecholamines. In analogy with polypeptide receptor systems (42, 43), the source of the altered membrane form of the \( \beta \)-adrenergic receptor would be a process of catecholamine-induced receptor endocytosis. Indeed, the fact that catecholamines induce a rapid, selective conversion of the \( \beta \)-receptor to a form that sediments during centrifugation with characteristics that clearly differentiate it from receptors bound to large fragments of plasma membrane is difficult to explain by a known mechanism other than endocytosis. Chuang and Costa (11), Chuang et al. (12), and Stadel et al. (13) have presented arguments in favor of the occurrence of agonist-induced endocytosis of the \( \beta \)-adrenergic receptor of frog erythrocytes. Intact cell binding assays with desensitized 1321N1 cells also suggest that, during short-term incubation with catecholamines, receptors are rapidly sequestered in a
localization that is inaccessible to membrane-impermeable \( \beta \)-adrenergic receptor radioligands. Similar results have been obtained using glioma and lymphoma cells (44-46).

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