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INTERACTIONS OF β-ADRENERGIC RECEPTORS WITH A MEMBRANE PROTEIN OTHER THAN THE STIMULATORY GUANINE NUCLEOTIDE-BINDING PROTEIN*

STEWART N. ABRAMSON,†‡ ROBERT G. L. SHORR§ and PERRY B. MOLINOFF‡‖

‡ Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104-6084, U.S.A.; and § Smith Kline & French Laboratories, Swedeland, PA 19479, U.S.A.

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Abstract—β-Adrenergic receptors on membranes prepared from rat lung, wild-type S49 lymphoma cells, and the adenylate cyclase-deficient variant of S49 lymphoma cells (cyc⁻) bind the agonist [^{3}H]hydroxybenzylisoproterenol ([^{3}H]HBI) with high affinity and this binding of [^{3}H]HBI can be inhibited by GTP. Membranes prepared from these tissues were incubated with the agonist [^{3}H]HBI or the antagonist [^{125}I]iodopindolol ([^{125}I]IPIN), labeled receptors were solubilized with digitonin, and the apparent molecular sizes of the ligand-bound receptor complexes were determined by high-performance size-exclusion chromatography. Results with all three tissues demonstrated that receptors labeled with [^{125}I]IPIN were retained by the size-exclusion columns longer than receptors labeled with [^{3}H]HBI. Thus, the apparent molecular size of soluble β-adrenergic receptors from rat lung, wild-type S49 cells, and cyc⁻ S49 cells was larger when receptors were occupied with an agonist rather than an antagonist. The results suggest that receptors, including those on cyc⁻ S49 cells, interact with a membrane protein, presumably a guanine nucleotide-binding protein. Since cyc⁻ S49 cells do not contain a functional stimulatory guanine nucleotide-binding protein, but do contain a functional inhibitory guanine nucleotide-binding protein, an interaction between β-adrenergic receptors and the inhibitory guanine nucleotide-binding protein may be responsible for the apparent increase in the molecular size of the receptor after occupation of the receptor with an agonist.

Receptors that increase the activity of the enzyme adenylate cyclase are thought to do so by activating the stimulatory guanine nucleotide-binding protein (N_s¶). Similarly, receptors that decrease the activity of adenylate cyclase are thought to do so by activating the inhibitory guanine nucleotide-binding protein (N_i). Interactions of receptors with guanine nucleotide-binding proteins can be investigated by examining the biochemical properties of the receptor as determined by size-exclusion chromatography or sucrose gradient centrifugation. For instance, when membrane preparations containing β-adrenergic [1–3], dopamine [4], or angiotensin [5] receptors were incubated with a radiolabeled agonist or antagonist, solubilized, and fractionated by size-exclusion chromatography, the molecular size of the receptors appeared to be larger when the receptors were labeled with an agonist rather than an antagonist prior to solubilization. In similar experiments, sucrose gradient centrifugation has been used to demonstrate that labeling of α-adrenergic receptors with an agonist prior to solubilization results in an increase in the apparent molecular size of the receptor [6].

The larger apparent molecular size of receptors after labeling with an agonist is thought to reflect the interaction of agonist-occupied receptors with a guanine nucleotide-binding protein. The observation that cholera toxin [^{32}P]ADP-ribosylated a 42,000-dalton protein that co-migrated through a size-exclusion column with agonist-occupied β-adrenergic receptors supports this hypothesis [2]. Moreover, N_s-like activity can be eluted by GTPγS from a wheat germ lectin-Sepharose column that has retained agonist-occupied β-adrenergic receptors, suggesting that it is possible to solubilize a stable complex composed of agonist, receptor, and N_s [7].

The radiolabeled agonist [^{3}H]hydroxybenzylisoproterenol ([^{3}H]HBI) has been used to investigate the properties of β-adrenergic receptors on membranes prepared from S49 lymphoma cells [8]. In both wild-type cells and the adenylate cyclase-deficient variant (cyc⁻), the apparent density of receptors determined with [^{3}H]HBI was less than that determined with the antagonist [^{125}I]iodopindolol ([^{125}I]IPIN), and the ability of receptors to bind [^{3}H]HBI was inhibited by the addition of GTP. These

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† Recipient of a Predoctoral Fellowship from the Pharmaceutical Manufacturers Association Foundation. Present address: Division of Pharmacology, School of Medicine, University of California-San Diego, La Jolla, CA 92093, U.S.A.

‖ Author to whom all correspondence should be addressed.

¶ Abbreviations: N_s and N_i, the stimulatory and inhibitory guanine nucleotide-binding proteins of adenylate cyclase, respectively; cyc⁻, the adenylate cyclase-deficient variant of S49 lymphoma cells; [^{3}H]HBI, [^{3}H]-hydroxybenzylisoproterenol; [^{125}I]IPIN, [^{125}I]iodopindolol; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); and HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

results are similar to those seen in other systems and are thought to result from an agonist-promoted interaction of β-adrenergic receptors with a guanine nucleotide-binding protein [9–11].

The net result of occupation of β-adrenergic receptors with an agonist is stimulation of the activity of adenylate cyclase. Therefore, β-adrenergic receptors presumably interact with N_s. Cyc^- S49 cells, however, do not express any of the functional activities of N_s [12–15]. The absence of a functional N_s in cyc^- S49 cells is probably due to the fact that cyc^- S49 cells do not express detectable amounts of the alpha subunit of N_s, as determined by immuno-blotting techniques, and they do not express detectable amounts of mRNA that codes for the alpha subunit of N_s, as determined by Northern analysis [16]. However, since cyc^- S49 cells contain a functionally active N_i [17–19], and purified β-adrenergic receptors have been shown to interact with purified N_i when the two proteins are reconstituted into lipid vesicles [20, 21], it is possible that results obtained in studies of the binding of [^{3}H]HBI to receptors on membranes prepared from cyc^- S49 cells reflect an interaction of β-adrenergic receptors with N_i.

To further characterize the proposed interaction of β-adrenergic receptors on membranes prepared from wild-type and cyc^- S49 cells with guanine nucleotide-binding proteins, the effect of incubating membranes with an agonist on the apparent molecular size of the receptor has been investigated. The results obtained suggest that membranes prepared from rat lung, wild-type S49 cells, and cyc^- S49 cells each contain a component, presumably a guanine nucleotide-binding protein, that is capable of forming a stable complex with the β-adrenergic receptor and increasing the apparent molecular size of the receptor. Since cyc^- S49 cells do not contain a functional N_s, but do contain a functional N_i, it is proposed that in these cells the guanine nucleotide-binding protein responsible for the observed effects is N_i.

MATERIALS AND METHODS

Materials. Racemic *p*-hydroxybenzyl[7-^{3}H]isoproterenol (7.7 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Carrier-free Na^{125}I (2200 Ci/mmol) was purchased from either New England Nuclear (Boston, MA) or Amersham (Arlington Heights, IL). (−)-Isoproterenol and digitonin were obtained from the Sigma Chemical Co. (St. Louis, MO). (−)-Pindolol was a gift of Dr. Günter Engel (Pharmaceutical Division, Sandoz Ltd, Basel, Switzerland), and (−)-propranolol was provided by Ayerst Research Laboratories (Princeton, NJ). TSK 4000SW (7.5 × 300 mm) and TSK 3000SW (7.5 × 300 mm) high-performance liquid chromatography (HPLC) size-exclusion columns were purchased from Beckman Instruments, Inc. (Altex Scientific Operations, Berkeley, CA).

Cell culture and preparation of cell membranes. S49 lymphoma cells were obtained from the cell culture facility of the University of California, San Francisco, CA. Cells were maintained in culture and membranes were purified as previously described [8, 12]. Membranes from rat lung were freshly prepared for each experiment as previously described [10]. The phenotypes of wild-type and cyc^- S49 cells were monitored periodically by assays of adenylate cyclase activity in the presence of isoproterenol and GTP, guanylyl-imidodiphosphate, NaF, $MnCl_2$, and forskolin. Although all of these agents increase the activity of adenylate cyclase in membranes prepared from wild-type S49 cells, only $MnCl_2$ and forskolin increase the activity of adenylate cyclase in membranes prepared from cyc^- S49 cells [8].

Preparation of soluble receptors labeled with radioligands. Digitonin (5%) was dissolved in water by heating at 90–95°, and the hot solution was filtered through a 0.22-μm Nucleopore filter. This solution was stored at 4° and was filtered through an activated SEP-PAC C_{18} cartridge (Waters Associates, Milford, MA) immediately before use. The antagonist [^{125}I]IPIN (2200 Ci/mmol), synthesized as previously described [22], and the agonist [^{3}H]HBI were used to label β-adrenergic receptors prior to solubilization of the receptors with digitonin. Membranes prepared from rat lung (0.5 to 1.5 mg/ml) or from S49 lymphoma cells (0.2 to 0.6 mg/ml) were incubated with [^{3}H]HBI (2–20 nM) in the presence or absence of 1.0 μM propranolol in buffer (final volume of 100 ml) containing 20 mM sodium-HEPES (pH 7.4), 1.0 mM $MgCl_2$, 0.5 mM pyrocatechol, and 0.5 mM ascorbic acid for 2 hr at 20°. Portions of the same preparations of membranes were diluted 10-fold and incubated in the same buffer (final volume of 20 ml) with [^{125}I]IPIN (50–500 pM) in the presence or absence of 50 μM isoproterenol for 2 hr at 20°. Membranes were collected by centrifugation at 20,000 *g* for 20 min at 4°. In some experiments, the pellets were gently washed with 20–30 ml of buffer containing 20 mM sodium-HEPES (pH 7.4) and 1.0 mM $MgCl_2$, and subjected to repeat centrifugation. The pellets were resuspended in 1.0 to 5.0 ml of buffer containing 1% digitonin, 20 mM sodium-HEPES (pH 7.4), and 1.0 mM $MgCl_2$, and incubated for 1 hr at 4°. Insoluble material was removed by centrifugation at 100,000 *g* for 1 hr. Supernatant fractions from the ultracentrifugation, containing soluble ligand-bound receptor complexes, were either fractionated immediately by size-exclusion HPLC or were frozen and applied to the HPLC columns the next day.

Specific binding of the radioligands was defined as the amount of radioligand bound in the absence of a competing ligand minus the amount of radioligand bound in the presence of an excess of a competitor. Specific binding of [^{3}H]HBI was defined with 1.0 μM propranolol, while specific binding of [^{125}I]IPIN was defined with 50 μM isoproterenol. Either isoproterenol or propranolol could be used to define specific binding, since both ligands inhibit the binding of [^{125}I]IPIN or [^{3}H]HBI to a similar extent [8]. The competing ligands used to define specific binding were of a chemical class different from that of the radioligands to minimize the possibility of inhibition of a common nonspecific component. To determine the amount of radioligand bound, free radioligand was separated from bound radioligand by filtration through glass-fiber filters [23]. The yield of soluble receptor complexes labeled with [^{3}H]HBI or [^{125}I]IPIN was approximately 10% of the receptors

Table 1. Stability of binding of [^{3}H]HBI and [^{125}I]IPIN to soluble receptors

Condition	Total	Nonspecific	Specific	Percent of control
	[^{3}H]HBI bound (fmol)			
0 min at 4°	42.2	7.0	35.2	100
13 hr at 4°	43.9	13.5	30.4	86
13 hr at 4° and then 1 hr at 37°	13.3	9.2	4.1	2
13 hr at 4° and then 1 hr at 4° with 1 mM GTP	36.9	9.4	27.5	78
	[^{125}I]IPIN bound (fmol)			
0 min at 4°	2.46	0.03	2.43	100
13 hr at 4°	1.79	0.03	1.75	72
13 hr at 4° and then 1 hr at 37°	0.02	0.02	0	0
13 hr at 4° and then 1 hr at 4° with 1 mM GTP	1.92	0.03	1.89	78

in the membrane that were labeled with either [^{3}H]HBI or [^{125}I]IPIN.

Protein content was determined by the method of Bradford [24], using bovine serum albumin as a standard. The counting efficiencies for ^{125}I and ^{3}H were 72 and 32% respectively.

Size-exclusion HPLC. The apparent molecular size of soluble ligand-bound receptor complexes was determined by size-exclusion HPLC at 4° through TSK 3000SW and TSK 4000SW columns linked in tandem. The mobile phase contained 0.1% digitonin, 100 mM Tris–HCl (pH 7.4), and 1.0 mM $MgCl_2$. The flow rate was maintained at 1.0 ml/min, and 0.5-ml fractions were collected. IgM (900,000 daltons), ferritin (540,000 daltons), catalase (240,000 daltons), aldolase (158,000 daltons), bovine serum albumin (67,000 daltons), hen egg albumin (45,000 daltons), chymotrypsinogen A (25,000 daltons), and cytochrome *c* (12,500 daltons) were used as standards. The variability of retention times for receptors labeled with either [^{3}H]HBI or [^{125}I]IPIN or for standard proteins was less than 0.2 min for replicate samples chromatographed on the same day.

RESULTS

Membranes prepared from rat lung were used as a normal control tissue in these studies. Rat lung was chosen for this purpose since it is a readily available source of tissue and since the binding of [^{3}H]HBI to receptors in this tissue has been characterized [10, 11]. Receptors on membranes prepared from rat lung were labeled with the agonist [^{3}H]HBI or the antagonist [^{125}I]IPIN and solubilized with digitonin. To determine whether the soluble ligand-bound receptor complexes were stable for the length of time required for size-exclusion chromatography, dissociation of the radioligands from the receptor was determined at various times after solubilization (Table 1). Less than 30% of either [^{3}H]HBI or [^{125}I]IPIN dissociated from the receptor after 13 hr at 4°. However, when the temperature was raised to 37°, more than 85% of the [^{125}I]IPIN and [^{3}H]HBI dissociated from the receptor within 1 hr. The ligand-bound receptor complexes were stable for more than 24 hr if frozen overnight at −20° (data not shown). Although GTP promotes the dissociation of [^{3}H]HBI from β-adrenergic receptors on membranes prepared from rat lung [10, 11], no effect of GTP on the dissociation of [^{3}H]HBI or [^{125}I]IPIN from soluble ligand-bound receptors was observed at 4° (Table 1).

Binding of the agonist [^{3}H]HBI to β-adrenergic receptors on membranes prepared from rat lung is thought to reflect the formation of a ternary complex composed of agonist, receptor, and a guanine nucleotide-binding protein [10, 11]. To provide biochemical evidence in support of this hypothesis, β-adrenergic receptors on membranes prepared from rat lung were labeled with the antagonist [^{125}I]IPIN, solubilized with digitonin, and fractionated by size-exclusion HPLC. Protein-bound [^{125}I]IPIN was retained by the gel and eluted as a single major peak (Fig. 1A). The peak of bound [^{125}I]IPIN was reduced in size when membranes were incubated with [^{125}I]IPIN in the presence of 50 μM isoproterenol prior to solubilization (Fig. 1A). The elution profile of receptors from the same preparation of membranes labeled with [^{3}H]HBI prior to solubilization was also determined (Fig. 1B). The two major peaks of bound [^{3}H]HBI were reduced in size when membranes had been incubated with [^{3}H]HBI in the presence of 1.0 μM propranolol prior to solubilization (Fig. 1B). The third peak of bound [^{3}H]HBI probably represented free [^{3}H]HBI since it eluted at the included volume of the gel and appeared to co-migrate with free [^{3}H]HBI (Fig. 1B). Comparison of the elution profiles of specifically bound [^{125}I]IPIN and [^{3}H]HBI (Fig. 1C) revealed that β-adrenergic receptors on membranes prepared from rat lung labeled with [^{3}H]HBI prior to solubilization had a larger apparent molecular size than receptors labeled with [^{125}I]IPIN prior to solubilization. In addition, receptors labeled with [^{3}H]HBI appeared to migrate with two distinct apparent molecular sizes as evidenced by the two major peaks of specifically labeled receptors.

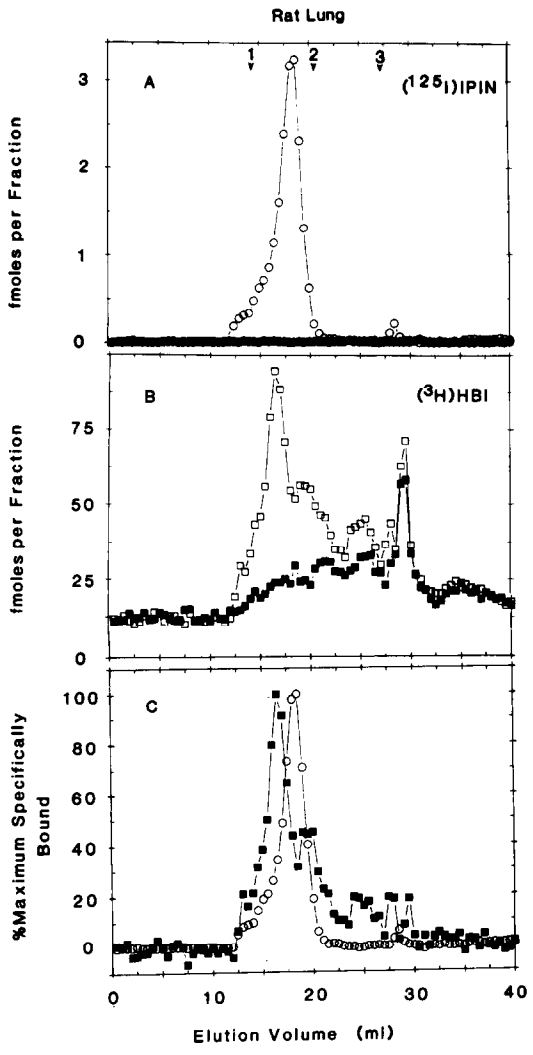

Fig. 1. Size-exclusion chromatography of receptors from rat lung. Membranes prepared from rat lung were incubated with [^{3}H]HBI (20 nM) in the presence or absence of 1.0 μM propranolol. A portion of the same preparation of membranes was incubated with [^{125}I]IPIN (240 pM) in the presence or absence of 50 μM isoproterenol. The membranes were collected by centrifugation and solubilized in buffer containing 1.0% digitonin. Insoluble material was removed by ultracentrifugation, and 0.5 ml of the supernatant fraction was chromatographed by size-exclusion HPLC at a flow rate of 1.0 ml/min. Two fractions were collected per min, and the amount of radioactivity in each 0.5-ml fraction was determined. (A) Elution profile of receptors labeled with [^{125}I]IPIN in the presence (●) or absence (○) of isoproterenol. (B) Elution profile of receptors labeled with [^{3}H]HBI in the presence (■) or absence (□) of propranolol. (C) Specific binding of [^{3}H]HBI (■) or [^{125}I]IPIN (○) to the β-adrenergic receptor was calculated by subtracting the amount of nonspecific binding from the amount of total binding. Protein standards were (1) IgM, (2) aldolase, and (3) cytochrome *c*. The results shown are representative of five separate experiments.

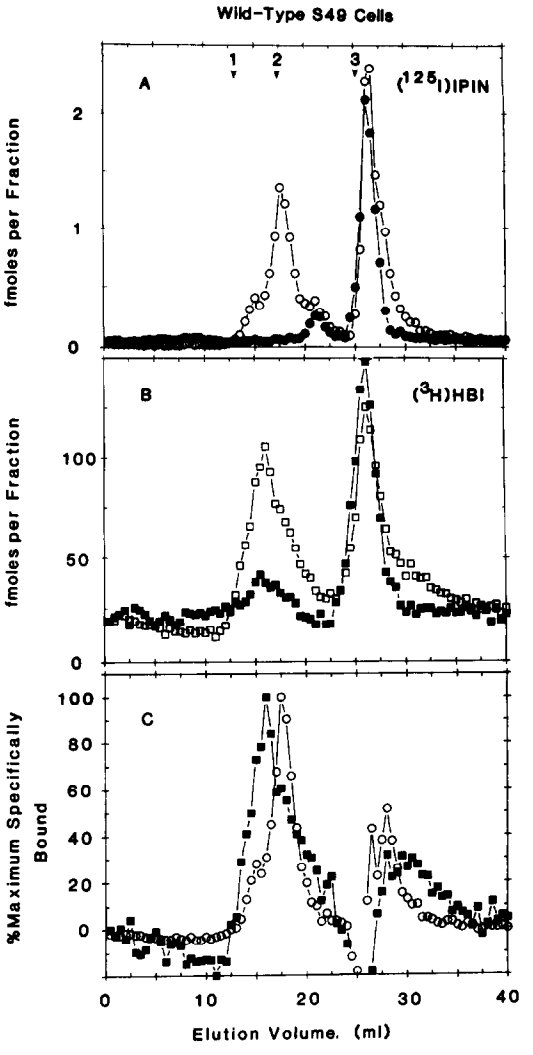

Fig. 2. Size-exclusion chromatography of receptors from wild-type S49 lymphoma cells. Membranes prepared from wild-type S49 cells were incubated with [^{3}H]HBI (20 nM) or [^{125}I]IPIN (500 pM) and processed as described in the legend to Figure 1. The results shown are representative of three separate experiments.

β-Adrenergic receptors on membranes prepared from wild-type S49 cells were also labeled with either [^{3}H]HBI or [^{125}I]IPIN, solubilized with digitonin, and fractionated by size-exclusion HPLC. [^{125}I]IPIN was included in the column and eluted as two major peaks (Fig. 2A). The first peak of [^{125}I]IPIN was decreased in size by co-incubation of membranes with isoproterenol during the incubation with [^{125}I]IPIN (Fig. 2A). The second peak of [^{125}I]IPIN eluted at the included volume of the gel, and probably represented free [^{125}I]IPIN (Fig. 2A). The same preparation of membranes was incubated with [^{3}H]HBI. [^{3}H]HBI was included in the column and eluted as two major peaks (Fig. 2B). The first peak of [^{3}H]HBI was decreased in size when membranes were labeled in the presence of propranolol (Fig. 2B). The second peak of [^{3}H]HBI probably represented free [^{3}H]HBI. Comparison of the elution profiles of specifically bound [^{3}H]HBI and [^{125}I]IPIN

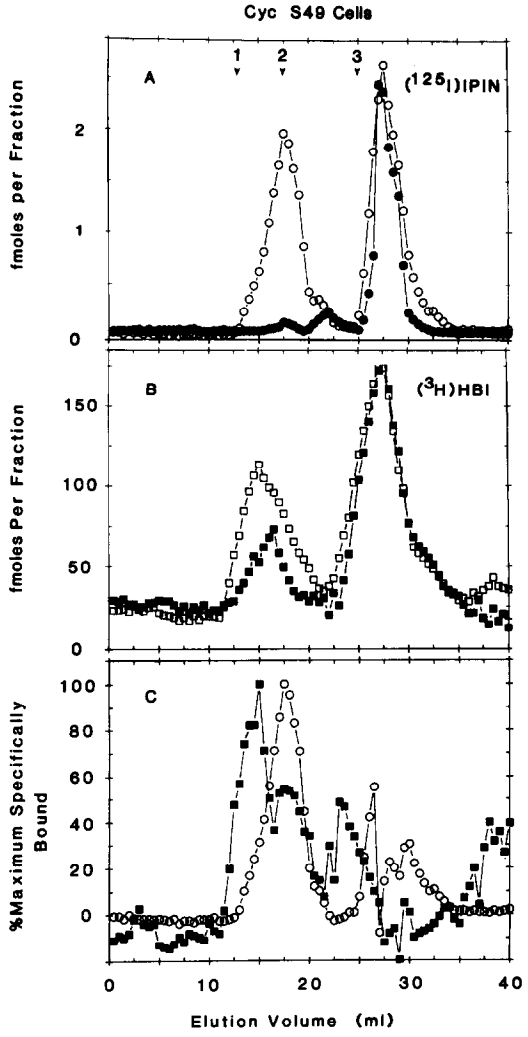

Fig. 3. Size-exclusion chromatography of receptors from cyc⁻ S49 lymphoma cells. Membranes prepared from cyc⁻ S49 cells were incubated with [^{3}H]HBI (20 nM) or [^{125}I]IPIN (500 pM) and processed as described in the legend to Figure 1. The results shown are representative of three separate experiments.

(Fig. 2C) revealed that receptors labeled with [^{3}H]HBI prior to solubilization had a larger apparent molecular size than receptors labeled with [^{125}I]IPIN prior to solubilization. In addition, the migration of receptors labeled with [^{3}H]HBI was asymmetric, and the peak of specifically bound [^{3}H]HBI had a shoulder on its trailing edge.

β-Adrenergic receptors on membranes prepared from cyc⁻ S49 cells can be assayed with either [^{3}H]HBI or [^{125}I]IPIN [8]. When membranes prepared from cyc⁻ S49 cells were labeled with [^{3}H]HBI or [^{125}I]IPIN, solubilized with digitonin, and fractionated by size-exclusion HPLC, the results were similar to those observed with receptors from membranes prepared from wild-type S49 cells (Fig. 3). [^{125}I]IPIN was retained by the gel and eluted as two major peaks (Fig. 3A). The first peak of [^{125}I]IPIN was decreased in size when membranes were incubated with [^{125}I]IPIN in the presence of 50 μM isoproterenol (Fig. 3A). The second peak of [^{125}I]IPIN probably represented free [^{125}I]IPIN. [^{3}H]HBI was also included in the column and eluted as two major peaks (Fig. 3B). Most of the radioactivity in the first peak represented specifically bound [^{3}H]HBI, while the second peak probably represented free [^{3}H]HBI (Fig. 3B). When the elution profiles of specifically bound [^{3}H]HBI and [^{125}I]IPIN were compared (Fig. 3C), it was clear that β-adrenergic receptors from cyc⁻ S49 cells had a larger apparent molecular size when the receptors were labeled with [^{3}H]HBI rather than with [^{125}I]IPIN prior to solubilization. In addition, receptors labeled with [^{3}H]HBI appeared to migrate with two distinct apparent molecular sizes as evidenced by the two major peaks of specifically labeled receptors (Fig. 3C).

DISCUSSION

When receptors on membranes prepared from rat lung, wild-type S49, or cyc⁻ S49 lymphoma cells were labeled with the agonist [^{3}H]HBI prior to solubilization, they had an apparent molecular size that was larger than receptors labeled with the antagonist [^{125}I]IPIN. The extent of nonspecific binding of [^{3}H]HBI was greater in membranes prepared from cyc⁻ S49 cells as compared with wild-type S49 cells [8], and this difference was maintained even after solubilization with digitonin. The reasons why one tissue exhibits more nonspecific binding of [^{3}H]HBI than another tissue are not readily apparent. However, differences in the apparent molecular sizes of receptors labeled with an agonist or an antagonist were similar in all three tissues, and were reproducible from experiment to experiment. Similar results have been reported for β-adrenergic receptors from frog erythrocytes [1] and rat reticulocytes [2, 3] and for dopamine [4] and angiotensin receptors [5].

Differences in the apparent size of agonist–receptor and antagonist–receptor complexes revealed by size-exclusion chromatography are thought to result from an agonist-induced interaction of receptors with another protein component of the adenylate cyclase system, presumably a guanine nucleotide-binding protein [1–5]. There is substantial evidence to support the hypothesis that agonists promote the interaction of β-adrenergic receptors with a guanine nucleotide-binding protein. For instance, guanine nucleotides cause a decrease in the apparent affinity of β-adrenergic receptors for agonists [9–12], β-adrenergic receptors stimulate a GTPase activity [25], and purified β-adrenergic receptors activate purified N_s [21, 26–28] and purified N_i [20, 21] when reconstituted into lipid vesicles. In addition, agonist-occupied β-adrenergic receptors have been shown to comigrate through size-exclusion chromatography with a 42,000-dalton protein that is [^{32}P]ADP-ribosylated by cholera toxin [2], and N_s-like activity is associated with receptors labeled with an agonist prior to solubilization [7]. Although differential mobility of agonist- and antagonist-occupied receptors through gel filtration chromatography probably reflects an agonist-promoted interaction between β-adrenergic receptors and a guanine nucleotide-binding protein, it is possible that it is the result of agonist-dependent changes in the amount of detergent bound to the

receptor, or to agonist-dependent changes in hydrophobic or ionic interactions of the receptor with the gel matrix.

Size-exclusion chromatography can be used to determine the apparent molecular size of a protein in solution [29]. However, proteins such as hen egg albumin, bovine serum albumin, and β-adrenergic receptors migrated through the size-exclusion columns used in these studies with apparent molecular sizes that were larger than expected. The apparent molecular sizes of detergent–receptor complexes labeled with either [^{125}I]IPIN or [^{3}H]HBI were greater than that of aldolase when determined by size-exclusion chromatography. Although its apparent molecular size is similar to that observed for the β-adrenergic receptor from other tissues as determined by size-exclusion chromatography [1–3], it is greater than the 40,000- to 62,000-dalton molecular weight of the receptor determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [30–32]. Association of different amounts of detergent with different proteins probably results in the unusually large apparent molecular sizes of proteins such as the albumins and the β-adrenergic receptor. Since the amount of digitonin associated with the β-adrenergic receptor cannot be assayed directly, size-exclusion HPLC was used primarily to compare the mobility of receptors labeled with either an agonist or an antagonist, rather than to determine the actual molecular size of the receptor.

In all three of the systems investigated, the elution profiles of receptors labeled with [^{3}H]HBI were either asymmetric or were composed of two peaks. Thus, [^{3}H]HBI appeared to label two forms of the receptor with different apparent molecular sizes. Similar results have been obtained in studies with membranes prepared from rat reticulocytes [2, 3]. These findings are consistent with the observation that agonists are capable of labeling two populations of β-adrenergic receptors with different affinities for agonists [9–12]. A complex composed of receptor and guanine nucleotide-binding protein is thought to bind agonists with high affinity, whereas the receptor alone is thought to bind agonists with a lower affinity.

Although recovery of soluble receptors that retained bound [^{3}H]HBI or [^{125}I]IPIN was relatively low (approximately 10% of the receptors in the membrane prior to solubilization), the ligands remained bound to the soluble receptors with little dissociation, provided that the samples were maintained at or below 4°. The possibility that the soluble ligand-bound receptors are not representative of the receptors in the membrane cannot be excluded due to the relatively low recovery of soluble ligand-occupied receptors. Thus, the relative ability of ligands to label both the larger and smaller forms of the receptor may not quantitatively reflect what occurs in the membrane.

Guanine nucleotides have been shown to accelerate the dissociation of [^{3}H]HBI from β-adrenergic receptors on membrane preparations, and this effect is thought to be mediated through a guanine nucleotide-binding protein [10, 11]. However, GTP did not cause dissociation of either [^{3}H]HBI or [^{125}I]IPIN from soluble ligand-bound receptors. It is possible that solubilization of the complex composed of agonist, receptor, and guanine nucleotide-binding protein results in alterations of the GTP-binding site, or that digitonin blocks the ability of GTP to bind to the guanine nucleotide-binding protein. Consistent with these hypotheses are the observations that β-adrenergic receptors are capable of stimulating the GTPase activity of N_s and the binding of GTPγS to N_s only when both proteins are removed from detergent and reconstituted into lipid vesicles [21, 26–28].

Absolute identification of a component(s) responsible for the apparent increase in the molecular size of β-adrenergic receptors will require isolation of the agonist-bound receptor complexes. However, since β-adrenergic receptors interact functionally with N_s, and the binding of [^{3}H]HBI to receptors on membranes prepared from rat lung [10, 11] and wild-type S49 cells [8] can be inhibited by GTP, the agonist-promoted increase in the apparent molecular size of β-adrenergic receptors from these tissues is probably due to an interaction of these receptors with N_s. Membranes prepared from cyc$^-$ S49 cells also appeared to contain a membrane protein that interacted with agonist-occupied β-adrenergic receptors, resulting in an increase in the apparent molecular size of the receptor. Cyc$^-$ S49 cells do not express a functionally active N_s [12–15]. The reason for this functional deficiency appears to be due to the fact that cyc$^-$ S49 cells do not make detectable amounts of the mRNA that codes for the alpha subunit of N_s [16]. However, since the binding of [^{3}H]HBI to receptors on membranes prepared from cyc$^-$ S49 cells can be inhibited by GTP [8], the agonist-promoted increase in the apparent molecular size of β-adrenergic receptors from this tissue could be due to an interaction of the receptor with another guanine nucleotide-binding protein, such as transducin, N_i, or G_0, a guanine nucleotide-binding protein isolated from bovine brain [33].

β-Adrenergic receptors have been shown recently to be capable of interacting with guanine nucleotide-binding proteins other than N_s. For instance, when purified β-adrenergic receptors were reconstituted with purified N_i, isoproterenol stimulated the incorporation of GTPγS into N_i and the GTPase activity of N_i [20, 21]. Although β-adrenergic receptors were reconstituted with similar concentrations of N_s or N_i, isoproterenol did not stimulate the GTPase activity of N_i to as great an extent as that of N_s [21]. However, since hydrolysis of GTP is thought to be the mechanism for termination of the activity of guanine nucleotide-binding proteins, and the relative affinity of β-adrenergic receptors for N_s or N_i cannot be determined from reconstitution with a single concentration of guanine nucleotide-binding protein, the physiological consequences of interactions between β-adrenergic receptors and N_i are as yet unknown. Since cyc$^-$ S49 cells express functional N_i [17–19], it is possible that association of β-adrenergic receptors on membranes prepared from cyc$^-$ S49 cells with N_i results in the observed increase in the apparent molecular size of the receptor. It is also possible that interaction of β-adrenergic receptors with N_i occurs in cells with a functional N_s, and that such an interaction is involved in attenuation of

receptor-stimulated adenylate cyclase activity, internalization of receptors, or some of the effects thought to be mediated by guanine nucleotide-binding proteins.

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