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Photoaffinity labeling of the β-adrenergic receptor from cultured lymphoma cells with [^{125}I]iodoazidobenzylpindolol: Loss of the label with desensitization

(S49 lymphoma cell line/down-regulation/receptor heterogeneity)

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ABSTRACT **The β-adrenergic antagonist [^{125}I]iodoazidobenzylpindolol ([^{125}I]IABP) specifically photolabeled two polypeptides in membrane preparations from wild-type (WT) and coupling protein-deficient cyc$^-$ cultured lymphoma cells. The molecular weights of the two polypeptides determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis were 65,000 and 55,000. They were labeled in a ratio of approximately 1:1. Pretreatment of intact WT or cyc$^-$ cells with 1.0 μM epinephrine for 15 min (desensitization) resulted in a greater loss of the 55,000 M_r polypeptide (40–60%) relative to the 65,000 M_r peptide (10–30% loss). An 18- to 24-hr pretreatment of WT cells with terbutaline (down-regulation) led to a >90% reduction of the photolabeling of both polypeptides, whereas a similar pretreatment of cyc$^-$ cells resulted in no further loss of labeled receptor than that observed after only a 15-min pretreatment with epinephrine. There was no indication of a change in the electrophoretic mobility of the [^{125}I]IABP-labeled receptors after either short- or long-term agonist pretreatment. These data provide direct evidence for heterogeneity of the β-adrenergic receptor in lymphoma cells. The differential loss of the [^{125}I]IABP labeling in the two polypeptides suggests a functional heterogeneity as well.**

The wild-type S49 lymphoma cell line (WT) and its coupling protein-deficient variant line (cyc$^-$) have proven to be an invaluable model system for the study of the mechanism of hormonal regulation (1) and desensitization of adenylate cyclase (2–4). We have demonstrated that short-term (0–60 min) pretreatment of both WT and cyc$^-$ cells with epinephrine leads to similar patterns of desensitization of adenylate cyclase in both cell lines (2, 4). Long-term pretreatment of the lymphoma cells with β-adrenergic agonists produced a greater than 90% depletion of epinephrine-stimulated adenylate cyclase and β-adrenergic receptor antagonist binding in WT membranes, whereas decreases in these parameters in cyc$^-$ membranes were no greater than after a 60-min pretreatment (2). Thus it appeared that while the lack of the subunit of the adenylate cyclase G/F coupling protein that is ADP-ribosylated by cholera toxin had no effect on short-term desensitization in cyc$^-$, G/F may have been required for down-regulation (2). The absence of a change in, or need for, the functional coupling protein complex during the early stage(s) of desensitization, combined with the near total loss of receptor stimulation of adenylate cyclase and β-antagonist binding with prolonged agonist pretreatment, strongly suggest that the receptor is either altered or occluded during agonist pretreatment.

Direct analysis of the β-adrenergic receptor structure either with or without desensitization has proven more difficult than the study of the coupling proteins, largely due to the lack of technology for identifying the receptor polypeptide(s) or for directly analyzing their function. Equilibrium binding studies of the β-adrenergic receptor in WT cells after *N*-ethylmaleimide alkylation of the membranes indicated there may be functional heterogeneity of the receptor, which further complicates analysis of the receptors in these cells (5).

Recently, Rashidbaigi and Ruoho (6) reported the synthesis of iodoazidobenzylpindolol (IABP), a β-adrenergic receptor photoaffinity ligand, and used the compound to determine the molecular weights of the β-adrenergic receptor ligand binding subunits in various erythrocyte membranes (7, 8). In the present study, we have utilized [^{125}I]IABP to determine: (*i*) the apparent molecular weights of the β-adrenergic receptor ligand binding subunits from cultured lymphoma cells (WT and cyc$^-$); (*ii*) the decreases that occur in the photolabeled β-adrenergic receptors with desensitization; and (*iii*) whether the decreases are accompanied by comparable changes in iodohydroxybenzylpindolol (IHYP) binding and epinephrine-stimulated adenylate cyclase activity.

MATERIALS AND METHODS

Materials. [^{125}I]IABP was synthesized at the same specific activity as carrier-free Na^{125}I (New England Nuclear) by the method described for the chemical synthesis (6). The source of other chemicals has been described (2, 7).

Cell Culture and Preparation of Membranes. WT and cyc$^-$ cells were grown in spinner culture as previously described (2). Cell incubations with (−)-epinephrine or terbutaline were carried out with 0.1 mM sodium ascorbate and 1.0 mM thiourea. Ascorbate/thiourea alone was added to the controls. Cell incubations were terminated by rapid cooling of the cells to 0–4°C. Epinephrine or terbutaline was then added to the control medium and plasma membrane fractions were prepared at 0–4°C by the procedure of Ross *et al.* (9) modified as follows: The crude particulate fraction was resuspended in 10% sucrose in 20 mM Hepes, pH 8.0/2 mM $MgCl_2$/1 mM EDTA (HME buffer) and then layered over 40% sucrose in HME and centrifuged at 80,000 × *g* for 80 min. The membranes floating over the 40% sucrose were collected, diluted with 5–6 vol of HME buffer, and centrifuged at 45,000 × *g* for 20 min. Membrane pellets were resuspended in HME buffer with 1.0 mM dithiothreitol, frozen, and stored at −70°C. Membranes were thawed prior to use and centrifuged at 48,000 × *g* for 15 min, and the pellets were resuspended in HME buffer for adenylate cyclase, [^{125}I]-

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Abbreviations: IHYP, iodohydroxybenzylpindolol; IABP, iodoazidobenzylpindolol; WT, wild-type S49 lymphoma cell line; cyc$^-$, coupling protein-deficient variant of S49; G/F, adenylate cyclase coupling proteins; HME, Hepes/$MgCl_2$/EDTA.

IABP labeling, and [^{125}I]IHYP binding assays. This wash reduced the dithiothreitol to a concentration that had no effect on the β-adrenergic receptor levels, adenylate cyclase, or [^{125}I]IABP labeling.

Adenylate Cyclase and [^{125}I]IHYP Binding Assays. The adenylate cyclase assays and [^{125}I]IHYP binding were carried out as described (2). cyc$^-$ membranes were reconstituted prior to assay by the procedure of Sternweis and Gilman (10), using cholate extracts of WT membranes. Comparisons of β-adrenergic receptor levels of control and epinephrine- or terbutaline-pretreated membranes (1–2 mg/ml) were carried out with [^{125}I]IHYP concentrations of 0.7–1.0 nM. We have detected no changes in the K_d of [^{125}I]IHYP binding after desensitization. Protein was determined either by using the procedure of Lowry *et al.* (11) with bovine serum albumin as the standard or by using the Bio-Rad protein reagent with α-globulin as the standard.

Photolabeling Procedure with [^{125}I]IABP. Membranes (1 mg/ml) in 0.2 ml of 10 mM Tris·HCl, pH 7.5, and 5 mM $MgCl_2$ (Tris buffer) were incubated in the presence and absence of 1.0 μM (−)-alprenolol with [^{125}I]IABP (1–2 nM) for 30 min at 30°C in the dark. The contents of the tubes were then cooled to 1°C in ice water, diluted quickly with 2 ml of ice-cold Tris buffer, and photolyzed immediately with an AH-6 high-pressure mercury lamp for 3 sec (7). The contents of the tubes were then diluted to 6 ml with Tris buffer and the membranes were collected by centrifugation at 200,000 × *g* for 45 min. The membrane pellets were denatured in 0.2 ml of a buffer containing 75 mM Tris·HCl at pH 6.8, 2.4% $NaDodSO_4$, and 10% glycerol. The amount of protein in each photolyzed sample was then determined (11). Prior to electrophoresis, 5 μl of 2-mercaptoethanol was added to each sample.

Slab Gel Electrophoresis of the Photolabeled β-Adrenergic Receptor. Electrophoresis of the photolabeled samples was performed according to the method of Laemmli (12) modified as described (7). The molecular weights of the receptor polypeptides were determined according to the method of Lambin *et al.* (13).

RESULTS

Affinity of IABP for the β-Adrenergic Receptor: IABP Inhibition of Adenylate Cyclase and Displacement of [^{125}I]IHYP. The effectiveness of a β-adrenergic receptor photoaffinity ligand is dependent in part on high affinity for the receptor and in part on the absence of nonspecific effects on the inhibition of adenylate cyclase activity. The data in Fig. 1 show that 10 μM unlabeled IABP fully inhibited 1.0 μM epinephrine-stimulated adenylate cyclase, and 50% inhibition was achieved at 45 nM. The calculated K_i for IABP was 2 nM when a value of 50 nM was used as the K_{act} for epinephrine as previously determined (2). There was no effect of 10 μM IABP on the prostaglandin E_1 (5.7 μM) stimulation of adenylate cyclase. We have also determined the K_d for unlabeled IABP binding to the WT membranes by displacement of [^{125}I]IHYP (data not shown). Direct binding experiments with [^{125}I]IABP in the dark do not provide a suitable ratio of specific to nonspecific binding. The calculated K_d for IABP binding was 0.94 nM. Thus, the agreement in the calculated affinity constants for IABP for adenylate cyclase inhibition and displacement of [^{125}I]IHYP was excellent.

Photoaffinity Labeling of the WT and cyc$^-$ β-Adrenergic Receptor with [^{125}I]IABP. WT and cyc$^-$ membranes were incubated in the presence of [^{125}I]IABP in the dark with or without (−)-alprenolol, photolyzed, and then examined by electrophoresis and autoradiography. Fig. 2 shows the autoradiograms of the gels and Fig. 3 the ^{125}I contents of the gel slices in the region of specific labeling (lanes A and C are WT controls and lanes E and G are the cyc$^-$ controls). [^{125}I]IABP specifically labeled two polypeptides in both WT and cyc$^-$ with apparent molecular weights of 65,000 and 55,000. The larger of the two peptides, R_1 (65,000), migrated just ahead of bovine serum albumin and the smaller peptide, R_2 (55,000), migrated just ahead of catalase. The range of molecular weights determined for WT and cyc$^-$ polypeptides (n = 4) are 63,500–67,200 (R_1) and 55,200–58,100 (R_2). The amount of specific [^{125}I]IABP incorporation was between 5% and 10%.

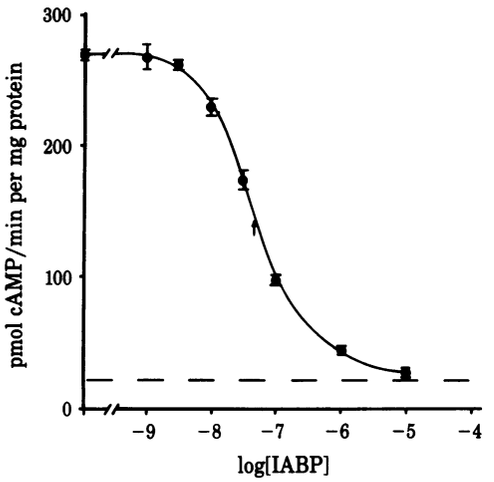

FIG. 1. Epinephrine-stimulated adenylate cyclase activity as a function of the concentration of IABP. Adenylate cyclase activity was determined as described (2) in the presence of 1.0 μM epinephrine and various concentrations of unlabeled IABP. The apparent K_i for IABP was determined from the formula $K_i = [K_{act}/([\text{epinephrine}] + K_{act})] \times I_{50}$, in which K_{act} is the activating constant for epinephrine and I_{50} is the concentration of IABP that inhibited 50% of the epinephrine-stimulated activity. The broken line indicates basal activity.

The two polypeptides were approximately equally labeled in both cell lines. This can be seen both in the autoradiograms, which show better resolution of the labeling pattern, and in the more quantitative radioactivity measurements of the sliced gels. In four experiments with cyc$^-$ [^{125}I]IABP in the 55,000 M_r band was 102 ± 13% (mean ± SD; n = 4) of that in the 65,000 M_r band, and in WT [^{125}I]IABP in the 55,000 M_r band was 100 ± 3.0% (n = 3) of that in the 65,000 M_r band. The ratio of polypeptide labeling was not affected by inclusion of the protease inhibitors benzamidine (25 mM) and phenylmethylsulfonyl fluoride (0.2 mM) or EDTA (5 mM) in the cell lysis buffer and through the entire membrane purification procedure (data not shown). However, equal labeling of the 55,000 and 65,000 M_r polypeptides was not always observed, as can be seen by comparing lanes E and G (Figs. 2 and 3), which are the long- and short-term desensitization controls, respectively. The reasons for variations in the ratios are unknown, although one possibility is the growth of the cells in medium containing horse serum, which is known to affect β-adrenergic receptor levels (14).

Several proteins were nonspecifically labeled by [^{125}I]IABP, in particular a 47,000 M_r protein and to a lesser extent proteins with molecular weights of approximately 84,000 and 99,000. None of these nonspecifically labeled proteins was reproducibly displaced by alprenolol, nor were they altered by desensitization. The amount of ^{125}I in the nonspecifically labeled bands was low relative to the specific labeling. In control experiments (data not shown) further demonstrating the specificity of IABP labeling, we have found that (*i*) the labeling of these two polypeptides was protected by the addition of an agonist, 4 μM isoproterenol; (*ii*) the specific [^{125}I]IABP labeling of WT was un-

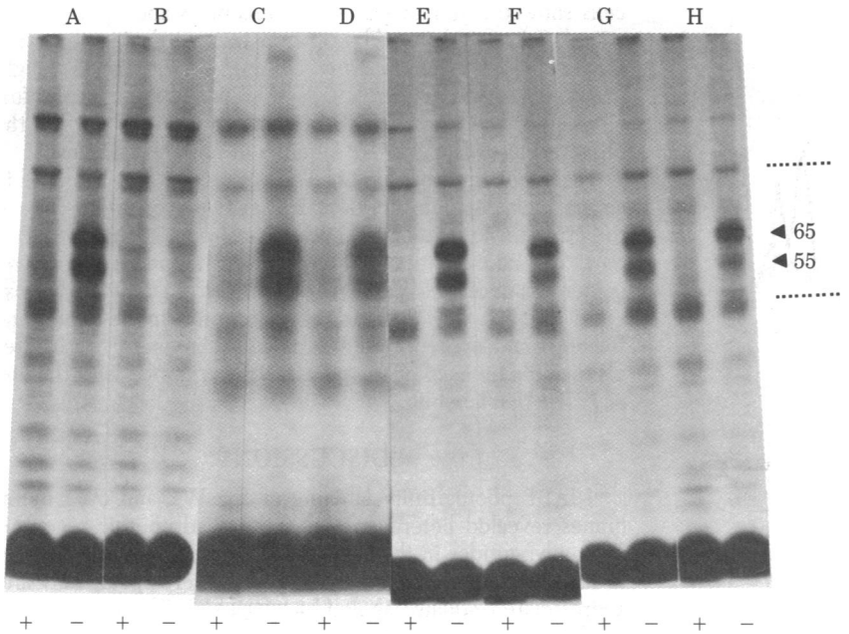

FIG. 2. Autoradiograms of the $NaDodSO_4$/polyacrylamide gel electrophoresis patterns of [^{125}I]IABP-photolabeled β-adrenergic receptors from WT and cyc$^-$. The membranes for lane pairs A, B, and E–H were incubated with 2 nM [^{125}I]-IABP (893 Ci/mmol; 1 Ci = 3.7×10^{10} Bq), the mixture was photolyzed, and then 120 μg of protein was applied to 7–14% linear gradient slab gels. The proteins were electrophoresed and autoradiographed. The membranes used in lane pairs C and D were incubated with 1.05 nM [^{125}I]-IABP at 935 Ci/mmol and the amount of protein placed on these gels was 84 μg. All of the membranes, lanes A–H, were incubated with (+) or without (−) 1.0 μM alprenolol as indicated below the gels. Time of development of all the autoradiograms was 20–24 hr. Ascorbate/thiourea was added to the media of the controls and the epinephrine- or terbutaline-treated cells. Controls were incubated for the same length of time as the treated cells. The autoradiograms shown were as follows: Lanes A–D, WT membranes; lanes E–H, cyc$^-$ membranes. Lanes A, C, E, and G, controls; lane B, terbutaline pretreatment for 18 hr; lane D, epinephrine for 15 min; lane F, terbutaline for 23 hr; lane H, epinephrine for 15 min. The portions of the gels between the dotted lines were sliced in 2-mm segments and the ^{125}I contents were measured (see Fig. 3). Molecular weights × 10^{-3} are indicated.

affected by the presence of bovine serum albumin at 1 mg/ml during the incubation and photolysis, consistent with the labeling being purely intramolecular (7, 15); and (*iii*) washing the membranes twice prior to photolysis did not affect the specific [^{125}I]IABP labeling pattern, although background label was reduced.

Effect of Agonist-Specific Down-Regulation on the [^{125}I]IABP Labeling, [^{125}I]IHYP Binding, and Adenylate Cyclase Activity. The data in Figs. 2 and 3, lanes B, show the [^{125}I]IABP labeling pattern of the WT membranes after an 18-hr incubation of the cells with the stable β-adrenergic agonist terbutaline (0.1 mM). Similar results were found when epinephrine was used for the long-term pretreatments, but the lability of the catecholamine necessitated repetitive additions. The 18-hr treatment of WT resulted in the nearly complete elimination (<5% of controls) of any specific [^{125}I]IABP labeling in the WT membranes. The epinephrine-stimulated adenylate cyclase of these WT membranes was specifically desensitized 89% by terbutaline, and the number of β-adrenergic receptor ligand binding sites measured by [^{125}I]IHYP binding was reduced 82%.

In contrast to WT, the 23-hr desensitization of cyc$^-$ with 0.1 mM terbutaline (Figs. 2 and 3, lanes E and F) resulted in approximately a 25–35% decrease in [^{125}I]IABP labeling of the 65,000 M_r polypeptide and a 40–60% decrease in the 55,000 M_r polypeptide. In this experiment the epinephrine-stimulated adenylate cyclase of reconstituted cyc$^-$ was desensitized 52% and the number of β-adrenergic receptor ligand binding sites determined by [^{125}I]IHYP binding was reduced by 39%.

Effect of Acute Desensitization on the [^{125}I]IABP Labeling of cyc$^-$ and WT. We have previously shown that a 30-min epinephrine pretreatment of intact cyc$^-$ and WT produced similar decreases in epinephrine-stimulated adenylate cyclase (2). To determine the effects of acute desensitization on the proteins specifically labeled by [^{125}I]IABP, cyc$^-$ and WT were pretreated for 15 min with 1.0 μM epinephrine. In the WT, labeling of the 55,000 M_r protein was decreased to a greater extent than that of the 65,000 M_r protein (Figs. 2 and 3, lanes C and D). The decreases in the photolabeling of both WT proteins were significant. This can be seen in Table 1, where the data from two experiments are summarized. The average decreases in the 65,000 and 55,000 M_r proteins were 28% and 62.5%, respectively.

In the cyc$^-$ cells, photolabeling was reduced about 60–70% in the 55,000 M_r polypeptide relative to the control (see Figs. 2 and 3, lanes G and H), whereas the labeling of the 65,000 M_r protein was just slightly, but not significantly, reduced. Similar results—that is, a greater decrease in the 55,000 M_r polypeptide relative to the 65,000—were also seen with a 2-hr desensitization of cyc$^-$ cells (data not shown).

[^{125}I]IHYP Binding and Adenylate Cyclase After Short- and Long-Term Hormone Pretreatment. Several groups have reported that changes in [^{125}I]IHYP binding occur subsequent to changes in epinephrine-stimulated adenylate cyclase in crude particulate preparations from WT cells during the rapid stage of desensitization (3, 16). To establish whether the changes observed with [^{125}I]IABP labeling of purified membrane fractions from cells pretreated with β-adrenergic agonists for either short

Table 1. Loss of the specific [^{125}I]IABP labeling of membranes after a 15-min epinephrine desensitization of intact WT cells

Exp.	Receptor protein	[^{125}I]IABP incorporation, fmol/mg		Decrease after Epi pretreatment, %
		Control	Epi pretreated	
1	65,000	25.3 ± 1.0	16.7 ± 0.97	34
	55,000	22.1 ± 0.19	8.4 ± 0.43	62
2	65,000	24.8 ± 0.50	19.5 ± 1.3	21
	55,000	23.9 ± 0	8.72 ± 0.51	64

Intact WT cells were pretreated with or without 1.0 μM epinephrine (Epi) for 15 min. Epinephrine was added to controls after the cells had been cooled to 0–4°C (2). Membranes were prepared, labeled with [^{125}I]IABP, and electrophoresed. The specific activity of [^{125}I]IABP was 935 Ci/mmol and its concentration was 1.05 nM. The amount of protein added to each gel was 84 μg. Gels were sliced, ^{125}I contents were measured, and the specific ^{125}I contents of the 65,000 M_r and 55,000 M_r proteins were totaled. Values shown are the means of the specific radioactivities of duplicate gels (± half the range). The specific activities of [^{125}I]IABP incorporation were calculated on the basis of the total protein added to the gel (84 μg).

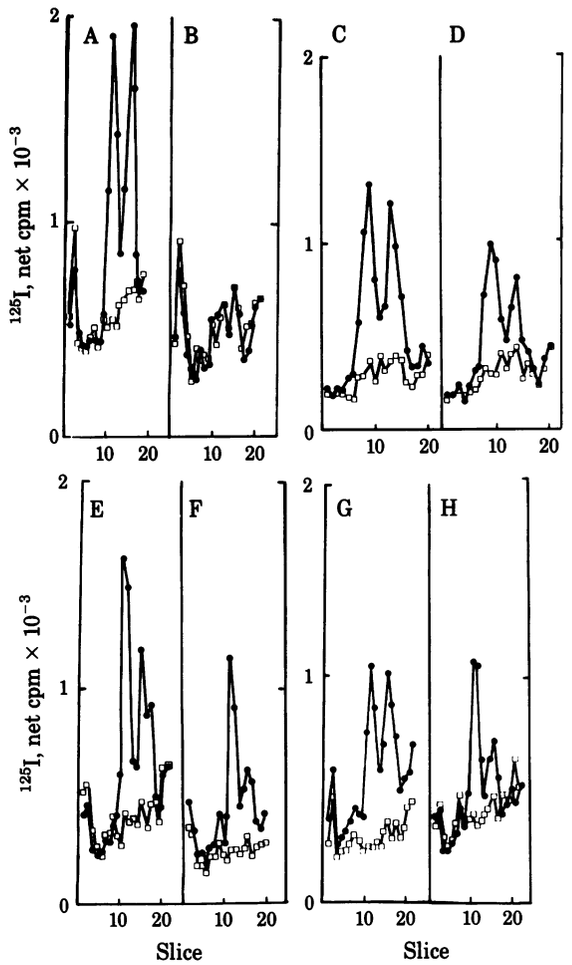

FIG. 3. ^{125}I profiles of the NaDodSO$_4$/polyacrylamide gels of the [^{125}I]IABP-photolabeled β-adrenergic receptors from WT and cyc$^-$. The description of the samples applied to the gels is given in the legend to Fig. 2. Membranes were incubated with (□) or without (●) 1.0 μM alprenolol. The portions of the gels between the dotted lines shown in Fig. 2 were sliced into 2-mm segments and ^{125}I contents were measured.

(15–30 min) or long (18–24 hr) times were also reflected in decreased [^{125}I]IHYP binding and receptor function (epinephrine-stimulated adenylate cyclase), the experiments summarized in Table 2 were performed. Fifteen- and 30-min epinephrine pretreatment of WT cells resulted in a 26 ± 7% and 27 ± 8% loss of receptor with a concomitant 37 ± 4% and 48 ± 6% loss of epinephrine-stimulated adenylate cyclase. cyc$^-$ cells showed a similar loss of the receptors and epinephrine-stimulated adenylate cyclase (reconstituted) during the rapid stage of desensitization. Thus, although we observed a slight dissociation of cyclase and [^{125}I]IHYP binding, the apparent loss of receptor was clearly significant and correlates well with the observed loss of [^{125}I]IABP labeling in both WT and cyc$^-$.

The long-term treatment of WT with terbutaline resulted in an average receptor loss of 94 ± 2% relative to controls, whereas the epinephrine-stimulated cyclase was reduced an average of 89 ± 1%. Similar treatment of cyc$^-$ resulted in an average receptor loss of 32 ± 4% and a 53 ± 1% loss of adenylate cyclase activity. Again, the apparent loss of receptors in the membrane fractions and epinephrine stimulated adenylate cyclase after comparable times of agonist pretreatment agreed with the loss of [^{125}I]IABP labeling.

DISCUSSION

[^{125}I]IABP photoaffinity labeling of the WT and cyc$^-$ membranes revealed heterogeneity of the β-adrenergic receptor. Two polypeptides in the cyc$^-$ and WT lymphoma membranes of molecular weights 65,000 and 55,000 were specifically and approximately equally labeled by [^{125}I]IABP. The specificity of the labeling, the good correlation with receptor levels determined by [^{125}I]IHYP binding, and the complete loss of both proteins in WT with chronic β-adrenergic receptor desensitization suggested that both polypeptides may contain ligand binding site of the β-adrenergic receptor. Whether or not the [^{125}I]IABP labeling represents the actual stoichiometry of the polypeptides containing β-adrenergic antagonist binding sites is unknown because, as with most photoaffinity labels (17), only a small percentage (5–10%) of the total number of β-adrenergic receptors was photolabeled.

Our evidence suggests that the [^{125}I]IABP is binding and labeling the 65,000 and 55,000 M_r polypeptides through an intramolecular mechanism, although we have not yet completely ruled out the possibility that [^{125}I]IABP is labeling a protein in close proximity to the ligand binding site. One candidate for nearest-neighbor labeling would be the 55,000 M_r subunit of G/F (18). However, it is unlikely that the 55,000 M_r protein labeled with [^{125}I]IABP in lymphoma cells is the 55,000 M_r subunit of the G/F protein, because it appears to be absent from cyc$^-$ cells (18, 19).

Table 2. Decrease in epinephrine-stimulated adenylate cyclase and [^{125}I]IHYP binding with acute and chronic desensitization

Cells	Pretreatment time	n	[^{125}I]IHYP binding, fmol/mg		Desensitization, %	Epinephrine-stimulated adenylate cyclase, pmol/min per mg		Desensitization, %
			Control	Treated		Control	Treated	
WT	15 min	5	332 ± 46	240 ± 36	26 ± 7	488 ± 70	316 ± 20	37 ± 4
	30 min	3	230 ± 52	160 ± 25	27 ± 8	542 ± 83	287 ± 27	48 ± 6
	18–24 hr	6	215 ± 56	28 ± 13	89 ± 4	263 ± 37	24 ± 9	93 ± 1
cyc$^-$	10 min	5	566 ± 79	436 ± 69	23 ± 4	112 ± 9	79 ± 7	31 ± 4
	15 min	6	444 ± 68	290 ± 74	38 ± 7	72 ± 2	46 ± 13	41 ± 2
	18–24 hr	4	401 ± 71	276 ± 44	30 ± 4	56 ± 7	28 ± 4	54 ± 3

Cells were pretreated with 1.0 μM epinephrine for 10–30 min. For the long-term incubations cells were treated with 0.1 mM terbutaline. Plasma membrane fractions were prepared and both the epinephrine (1.0 μM) stimulation of adenylate cyclase and the number of receptors were determined. cyc$^-$ membranes were reconstituted and assayed as described (2). There were no significant changes in basal, prostaglandin E_1-, or NaF-stimulated activities with the various pretreatments of either cyc$^-$ or WT. Binding was measured at an [^{125}I]IHYP concentration of 0.7–1.0 nM. Adenylate cyclase activities are presented with basal activity subtracted. Mean basal activities for WT and cyc$^-$ were 26.3 ± 2.2 and 5.1 ± 0.6 pmol/min per mg of protein. The percent desensitization is the average of the percent desensitizations of each experiment. All values are the means ± SEM.

Our observations on [^{125}I]IABP labeling of the β-receptor after agonist pretreatment can be summarized as follows: (*i*) there is no observable change in the mobility of the labeled polypeptides; (*ii*) labeling of the 55,000 M_r protein was preferentially lost with short-term agonist pretreatment; and (*iii*) labeling of both polypeptides was lost with prolonged agonist pretreatment of WT but not of cyc$^-$. These observations are consistent with several possibilities. One is that the 65,000 M_r polypeptide is a precursor of the 55,000 M_r species that desensitizes by at least a two-stage process: $R_1 \rightarrow R_2 \rightleftharpoons R' \rightarrow R''$, in which R′ is the rapidly and reversibly desensitized form of the receptor and R″ is the down-regulated state (3). The absence of down-regulation from cyc$^-$ cells could reflect a dependency on the functional G/F for the $R' \rightarrow R''$ conversion. A second possibility is that there are two populations of the β-adrenergic receptor that desensitize at different rates, desensitization of the 55,000 M_r protein proceeding faster than that of the 65,000 M_r species. Again, to account for the lack of down-regulation in cyc$^-$ cells one must postulate a G/F-dependent stage for the desensitization of each β-receptor population. A third plausible scheme is that both polypeptides are subunits of a multisubunit receptor complex. The complex could be modified during desensitization such that the [^{125}I]IABP labeling of the 55,000 M_r polypeptide is preferentially decreased. Haga *et al.* (20) have determined the molecular weight of the Lubrol-solubilized lymphoma β-receptor by sucrose gradient sedimentation and molecular sieving. The value reported was 75,000 ± 10,000, the large range being attributable to the rather substantial correction for detergent binding. The lower limit of this value agrees well with the molecular weight of the larger [^{125}I]IABP-labeled subunit, making it unlikely that the β-receptor is a combination of the 55,000 and 65,000 M_r polypeptides in Lubrol.

The lack of any observable change in the molecular weight of the receptor after short- or long-term desensitization of the lymphoma cells is in contrast to the results of Stadel *et al.* (21). These investigators found an increase in the apparent molecular weights of the two photoaffinity-labeled turkey erythrocyte β-receptor polypeptides after a 4-hr pretreatment of the erythrocytes with 10 μM isoproterenol. There are other differences between the turkey erythrocytes and the lymphoma cells; notably, prolonged catecholamine pretreatment of turkey erythrocytes does not lead to the loss of receptors (down-regulation) as measured by antagonist binding, and cAMP analogues such as 8-bromo-cAMP induce a desensitized state in erythrocytes similar to that produced by catecholamines (22). By comparison, antagonist binding was almost eliminated in WT after prolonged catecholamine pretreatment. Further, three lines of evidence indicate that cAMP *per se* plays no role in the epinephrine desensitization of the lymphoma cells: (*i*) the acute phase of desensitization proceeds in cyc$^-$ in the complete absence of measurable cAMP, (*ii*) forskolin, which increases cAMP levels in intact WT, does not desensitize the cells (23), and (*iii*) catecholamine-induced desensitization proceeds normally in the variant of WT lacking cAMP-dependent protein kinase (24). We conclude that desensitization and down-regulation of the lymphoma cells must occur independent of cAMP or cAMP-dependent protein kinase. On the other hand, desensitization of the turkey erythrocyte may occur by cAMP-dependent phosphorylation of the receptor.

It has been shown previously that there is a significant lag in the loss of β-adrenergic receptors relative to the loss of adenylate cyclase activity in the crude particulate preparations from WT cells and astrocytoma cells (3, 16). We have found that a 15-min epinephrine desensitization of cyc$^-$ and WT leads to highly significant decreases in the β-adrenergic receptor population in plasma membrane fractions, although, in agreement with the previous studies, the decreases are less than the changes in epinephrine-stimulated adenylate cyclase. Harden *et al.* (25) have shown that the rapid phase of desensitization in astrocytoma cells is accompanied by the appearance of receptors in "light" vesicle fractions that are nearly devoid of adenylate cyclase activity. Our use of partially purified plasma membrane fractions may lead to the loss of desensitized receptors to a light vesicle fraction, resulting in the loss of [^{125}I]IHYP binding and [^{125}I]IABP labeling we observe. Further studies involving photolabeling of the receptor polypeptides in crude particulate fractions and various vesicle populations should resolve these important questions.

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