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Biochemistry

Monoclonal antibodies to β-adrenergic receptors: Use in purification and molecular characterization of β receptors

(mouse hybridomas/monoclonal antibody affinity columns/receptor classification/β-receptor subunits)

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ABSTRACT We have developed four hybridomas that produce monoclonal antibodies to the turkey erythrocyte β_1-adrenergic receptor and one hybridoma that produces a monoclonal antibody to the calf lung β_2 receptor. Splenic lymphocytes from BALB/c mice immunized with partially purified turkey erythrocyte β_1 receptors or calf lung β_2 receptors were fused with the mouse myeloma line SP2/0-Ag14 to yield hybridoma cultures producing β receptor monoclonal antibodies of the IgG class. The anti-turkey erythrocyte β receptor antibodies precipitated partially purified β receptors and inhibited adrenergic ligand binding. In contrast to autoantibodies to β_2-adrenergic receptors [Venter, J. C., Fraser, C. M. & Harrison, L. C. (1980) *Science* 207, 1361–1363] which do not crossreact with cardiac β_1 receptors, monoclonal antibody FV-104 directed against the adrenergic ligand binding site of turkey erythrocyte β receptors crossreacted equally with calf liver and lung β_2 receptors as well as calf heart β_1 receptors. These data suggest that some molecular homology exists between β-adrenergic receptors of substantially diverse pharmacological classes. We utilized the monoclonal antibodies in the final stage of turkey erythrocyte β_1 receptor purification. Turkey erythrocyte β receptors eluted from FV-104 monoclonal antibody affinity columns with NaDodSO$_4$ appeared as three components of M_r 70,000, 31,000, and 22,000 on NaDodSO$_4$/polyacrylamide gels. Iodination of material eluted from immunoaffinity columns with propranolol demonstrated the existence of only a single component (M_r, 70,000), indicating that the turkey erythrocyte β_1 receptor can be purified to homogeneity.

Advances in the purification and molecular characterization of hormone and neurotransmitter receptors have been hindered in part by the extremely low tissue concentrations of physiologically relevant receptor molecules. With the exception of the nicotinic acetylcholine receptor, progress in receptor isolation has been largely dependent on immunological discoveries. Autoantibodies to insulin receptors, which have been identified in certain insulin-resistant diabetics, have been valuable tools in elucidating the subunit structure of the insulin receptor (1, 2). The solubilization and characterization of mammalian β-adrenergic receptors (3) permitted the detection of autoantibodies to β_2-adrenergic receptors in serums of patients with asthma or allergic respiratory disease (4, 5). These autoantibodies which interact with lung β_2 receptors but not cardiac β_1 receptors (4) have been utilized in this laboratory to purify calf lung β-adrenergic receptors (6). However, the impurity and low titers of human β-receptor autoantibodies (4) as well as the probable polyclonal nature of the autoantibodies may limit their utility.

With the use of recently devised techniques for the hybridization of antibody-producing lymphocytes and myeloma cells, pure monoclonal antibodies directed to single antigenic determinants can be prepared from small quantities of a complex mixture of antigens (7–9). The production of monoclonal antibodies to receptor molecules is therefore feasible, and these unique reagents will allow receptor purification as well as the study of receptor structure and function (3, 4, 10, 11).

We report here on the production of hybridoma clones, developed from mouse myeloma and mouse splenic lymphocytes, that produce monoclonal antibodies to β-adrenergic receptors. The antibodies precipitate solubilized β-adrenergic receptors and inhibit adrenergic ligand binding to β receptors of apparently unrelated species and tissues, indicating that some degree of molecular homology exists between pharmacologically unrelated β receptors.

We have utilized the anti-β-receptor monoclonal antibodies covalently coupled to Sepharose 4B as the final step in turkey erythrocyte β receptor purification. Material specifically eluted from monoclonal antibody affinity columns and analyzed on NaDodSO$_4$/polyacrylamide gels demonstrates a primary molecular weight of 70,000 for the turkey erythrocyte β-adrenergic receptor, with a possible subunit of molecular weight 31,000.

MATERIALS AND METHODS

Polyethylene glycol 1000, aminopterin, hypoxanthine, and thymidine were from Sigma; Dulbecco's modified Eagle's medium and fetal bovine serum were from GIBCO; Pristane mineral oil was from Aldrich; digitonin was from Fisher (lot 796678); Ampholines were from LKB; and mouse immunoglobulins and antisera were from Miles. Other materials were obtained as described (3). SP2/0-Ag14 myeloma cells were kindly provided by R. Suddith.

Sources of β-Adrenergic Receptors. β-Adrenergic receptors were solubilized from turkey erythrocyte ghosts with 0.5% digitonin (12, 13) or from calf lung membranes (6) with 0.5% Triton X-100 and partially purified by gel exclusion chromatography and preparative isoelectric focusing as described (6, 13).

Immunizations and Cell Hybridizations. Six-week-old BALB/c mice received intraperitoneal injections of partially purified β-adrenergic receptors emulsified in an equal volume of Freund's incomplete adjuvant. Booster intraperitoneal injections without adjuvant were administered 4 weeks later, and mice were sacrificed 3–4 days after the booster immunizations.

Mouse spleen cells were fused with mouse myeloma SP2/0-Ag14 (SP2) cells according to the method of Gefter *et al.* (14). SP2 cells do not synthesize immunoglobulin chains (15). The fusion of spleen cells (1×10^8) with myeloma cells (1×10^8) in the logarithmic phase of growth was accomplished by addition of 1 ml of warm 50% polyethylene glycol 1000 (PEG 1000). The cell suspension was slowly diluted with 12 ml of Dulbecco's

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Abbreviation: IHYP, [^{125}I]iodohydroxybenzylpindolol.

modified Eagle's medium and centrifuged; the cells were resuspended in 20 ml of medium containing 20% fetal bovine serum, thymidine, and hypoxanthine (HT medium). One-tenth milliliter of the cell suspension which contained 10^6 total cells was plated into each well of two 96-well microtiter plates containing 10^4 nonimmune BALB/c spleen cells per well as a feeder layer. On the day after cell fusion, 0.1 ml of 2X HAT selective medium (hypoxanthine/aminopterin/thymidine) (16) was added to each well. Six to seven half-replacements of the selective medium were performed during the next 3 weeks. At about day 21 after the fusion, supernates from the wells containing hybridomas were assayed for anti-β-adrenergic receptor antibodies with an indirect immunoprecipitation assay (see below). Cells from the positive wells were cloned by limiting dilution and grown as ascites tumors in BALB/c mice primed with Pristane (17).

Characterization of Monoclonal Antibodies to β-Adrenergic Receptors. Hybridomas producing monoclonal antibodies to β-adrenergic receptors were grown in culture media containing 2 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{3}H]leucine and [^{3}H]methionine. Culture supernates were analyzed on 5% polyacrylamide isoelectric focusing gels over a pH range of 4 to 10 (18).

Indirect Immunoprecipitation Assay. Culture medium or ascites fluid was assayed for monoclonal antibodies by incubation of samples with partially purified turkey erythrocyte β-adrenergic receptors (specific activity, 1.6–5 pmol/mg of protein) for 18 hr at 4°C. Precipitation of receptor–monoclonal antibody complexes was accomplished by addition of rabbit anti-mouse IgG. Specific binding of [^{125}I]iodohydroxybenzylpindolol (IHYP) was recoverable in the immune precipitates and was proportional to the amount of receptor lost from the supernate. However, the immune complexes complicated the receptor assays. Therefore, the concentration of β-adrenergic receptors remaining in solution after immunoprecipitation was quantitated by labeling receptors with IHYP, a high affinity β receptor antagonist, in the presence or absence of 10 μM *l*-propranolol followed by precipitation of labeled soluble β receptors with 15% polyethylene glycol (3).

Culture media from hybridomas obtained from lung β receptor-immunized mice was screened for monoclonal antibodies to calf lung β receptors by using the indirect immunoprecipitation assay (4).

Effect of Monoclonal Antibodies on IHYP Binding to Soluble and Membrane-Associated β-Adrenergic Receptors. Serial dilutions of culture medium or ascites fluid containing monoclonal antibodies to turkey erythrocyte β receptors were preincubated with soluble β-adrenergic receptors from turkey erythrocytes, turkey erythrocyte ghosts, or purified plasma membranes from calf heart, liver, or lung for 60 min at 30°C prior to the determination of IHYP-specific binding as described for soluble or membrane associated β-adrenergic receptors (3, 4). Protein concentrations were adjusted to provide the same concentration of β-adrenergic receptors and antibody for each experiment. Phosphate-buffered saline containing mouse IgG at identical protein concentrations served as a control for any nonspecific effects of immunoglobulins on IHYP binding.

Purification of Turkey Erythrocyte β-Adrenergic Receptors with Monoclonal Antibody Affinity Columns. Purified monoclonal antibody FV-104 was prepared by ammonium sulfate precipitation (45% saturation) of ascites fluid followed by dialysis against 10 mM sodium phosphate buffer (pH 8) or by preparative isoelectric focusing in sucrose density gradients (0–50%) containing 1% Ampholines (pH 3–10) for 16 hr at 4°C (constant power, 15 W; maximal voltage, 1600 V). Antibody-containing fractions (identified by double immunodiffusion with anti-mouse IgG) were pooled and dialyzed overnight against 10 mM sodium phosphate buffer (pH 8). FV-104 IgG and control mouse IgG were coupled to CNBr-activated Sepharose 4B (5 mg of protein per ml of gel), and affinity columns were prepared in Eppendorf pipet tips (17) or ISCO polypropylene columns. Turkey erythrocyte ghosts were surface labeled with [^{131}I]iodine by using lactoperoxidase (19), and β-adrenergic receptors were solubilized from turkey erythrocyte membranes with 0.5% digitonin and partially purified by preparative isoelectric focusing. Samples containing ^{131}I-labeled turkey erythrocyte β receptors were applied to monoclonal antibody affinity columns or incubated with monoclonal antibody Sepharose. The Sepharose was then washed with phosphate-buffered saline, and receptors were eluted from the antibody with buffer containing 1% $NaDodSO_4$, 2.5% mercaptoethanol, and 5% glycerol (17). The eluate was characterized by electrophoresis on 10% polyacrylamide gels in $NaDodSO_4$ (18).

RESULTS

Preparation and Characterization of Hybridomas. Four hybridomas secreting monoclonal antibodies directed against turkey erythrocyte β receptors were derived from a cell fusion producing hybridomas in only 8 of 196 wells. In contrast, the hybridoma secreting an anti-calf lung β receptor antibody was the product of a cell fusion that generated hybridomas in 72 of 196 wells. Each clone described in Table 1 originated in a separate well of the microtiter plates, and further cloning by limiting dilution was performed to ensure antibody homogeneity. Each anti-β-receptor antibody was determined to be an IgG molecule by double immunodiffusion against subclass-specific rabbit anti-mouse immunoglobulins (not shown). Properties of the five anti-β-receptor monoclonal antibodies are summarized in Table 1.

Due to the greater concentration of anti-β receptor antibodies

Table 1. Anti-β-receptor monoclonal antibody-producing hybridomas

	FV-101	FV-102	FV-103	FV-104	FV-601
Immunoglobulin	IgG2a	IgG	IgG1	IgG1	IgG
pI	7.8	7.3	7.7	7.5	7.2
β-Receptors (fmol) precipitated at a 1:10 dilution of:					
Culture medium*	0.20	0.16	0.06	0.07	0.61
Ascites fluid†	—	—‡	0.85	0.30	—
% inhibition of IHYP binding to homologous receptors with a 1:10 dilution of:					
Culture medium§	16	33	33	14	0
Ascites fluid¶	—	—‡	—	34	—

The antigen was turkey erythrocyte β receptor for all clones except FV-601, for which it was calf lung β receptor.

* Mean from three separate experiments with soluble turkey erythrocyte β receptors (2 fmol per assay) or soluble calf lung β receptors (1.5 fmol per assay).

† Mean from three separate experiments with partially purified turkey erythrocyte β receptors (2 fmol per assay).

‡ Cells of clone FV-102 died soon after cloning, and characterization of these antibodies could not be completed.

§ Mean from three separate experiments with turkey erythrocyte membranes (0.8 fmol of receptor per assay) or calf lung membranes (1.6 fmol of receptor per assay).

¶ Mean from two separate experiments with turkey erythrocyte ghosts (1.6 fmol of receptor per assay).

obtained with ascites tumors grown in BALB/c mice, all additional characterizations of monoclonal antibody–receptor interactions were performed with ascites fluid. Ascites development required between 2 and 6 weeks. In some mice, ascites accumulation was complicated by the formation of large (>1 cm) solid tumors; at the time of ascites fluid recovery most mice contained multiple small tumors (<2 mm). Recovery of ascites fluid ranged from 0.2 to 7 ml of fluid per mouse. Hybridoma FV-104 was most abundant and therefore monoclonal antibody FV-104 was utilized in the majority of the studies. Ascites tumors have been serially passaged for more than 9 months without apparent change in antibody properties.

Effect of Monoclonal Antibodies on Adrenergic Ligand Binding to Soluble and Membrane Associated β-Adrenergic Receptors. In order to assess whether monoclonal antibody FV-104 (screened by immunoprecipitation, see below) to turkey erythrocyte β-adrenergic receptors was directed against a determinant in or near the ligand binding site, the effect of the antibodies on IHYP-specific binding to turkey erythrocyte ghosts was determined. Monoclonal antibody FV-104 inhibited the specific binding of IHYP to β receptors in turkey erythrocyte ghosts in a concentration-dependent manner compared to control mouse IgG ($P < 0.001$; Student's t test) (Fig. 1A). Antibody FV-104 was a more-potent inhibitor of IHYP binding to soluble turkey erythrocyte β receptors than to the same receptors in a membrane bound state. These data might be explained by a reduction in steric hindrance for antibody binding to the soluble receptor or could be due to a slight reduction in affinity of the soluble β receptor for IHYP (13).

In contrast to β receptor autoantibodies which affect ligand binding to calf and canine lung and human placental β_2-adrenergic receptors but not to canine cardiac β_1 receptors (3), monoclonal antibody FV-104 displayed essentially equal crossreactivity with calf lung, liver, and cardiac β-receptors (Fig. 1B). Preincubation of monoclonal antibody FV-104 with calf lung, liver, or heart membranes containing equivalent β receptor concentrations (1.6 fmol per assay) resulted in a concentration-dependent inhibition of IHYP-specific binding identical to that found with turkey erythrocyte β receptors.

Monoclonal antibody FV-104 binding to β-adrenergic receptors was directly competitive with IHYP as determined by the inhibition of IHYP-specific binding observed over a range of IHYP concentrations (twice, equal to, and half the IHYP K value for the indicated receptor) in the presence of identical serial dilutions of monoclonal antibody FV-104 (Fig. 2). From data obtained with calf lung, liver, and cardiac and turkey erythrocyte β receptors, it was calculated that monoclonal antibody FV-104 has an apparent affinity for β receptors in the range of 0.2–1 μM.

β-Adrenergic Receptor Immunoprecipitation. Monoclonal antibodies FV-103 and FV-104 precipitated β-adrenergic receptors in a concentration-dependent fashion (Fig. 3). In order to validate the receptor precipitation assay, we demonstrated that the loss of β receptors from solution as a consequence of antibody binding resulted in the appearance of β receptors in the immune precipitate. In addition, with the use of biosynthetically labeled monoclonal antibodies we demonstrated complete precipitation of monoclonal antibodies by anti-mouse IgG at each antibody dilution used. These data indicate that quantitation of receptors remaining in solution is not complicated by residual monoclonal antibody inhibiting ligand binding. The titers and shape of the immunoprecipitation curves suggest that the two monoclonal antibodies differ in their affinity for turkey erythrocyte β receptors.

Monoclonal Antibody Affinity Columns and β Receptor Purification. The specific and quantitative precipitation of β receptors by the monoclonal antibodies together with the relatively low affinity of the antibody for the receptor suggested that these antibodies might be ideal reagents for affinity purification of β receptors.

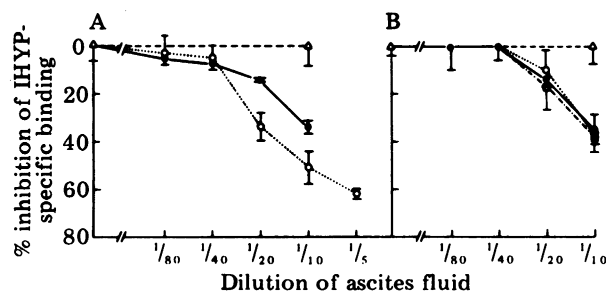

FIG. 1. Inhibition of IHYP-specific binding to soluble and membrane-associated β-adrenergic receptors by monoclonal antibody FV-104. Turkey erythrocyte ghosts (*A*, solid circles), partially purified turkey erythrocyte β receptors (*A*, open circles), purified calf lung membranes (*B*, open circles), purified calf heart membranes (*B*, solid circles), or purified calf liver membranes (*B*, open boxes) at protein concentrations required to provide 1.6 fmol of β receptor per assay were preincubated with serial 1:2 dilutions (in 20 mM $NaPO_4$/2 mM $MgSO_4$, pH 7.4) of ascites fluid containing monoclonal antibody FV-104 (17 mg of protein per ml) for 60 min at 30°C in a final volume of 100 μl. IHYP-specific binding to membranes was determined by incubating samples with saturating (2 times the K value) concentrations of IHYP in the presence and absence of 10 μM *l*-propranolol for 30 min at 30°C, diluting samples with 1.25 ml of filtration buffer [20 mM KPO_4/1 mM $MgSO_4$/0.1 mM (±)-propranolol], immediately filtering samples through 2.4-cm Whatman glass-fiber filters and washing filters with 25 ml of wash buffer at room temperature. IHYP-specific binding to soluble turkey erythrocyte β receptors was assayed as described in the legend of Fig. 3. Serial dilutions of mouse IgG (17 mg of protein per ml) served as the control for each receptor system (*A* and *B*, open triangles). IHYP-specific binding to soluble and membrane bound β-adrenergic receptors in the presence of control mouse IgG did not differ from IHYP-specific binding in the absence of immunoglobulins. The values are mean ± SEM of duplicate determinations from two separate experiments.

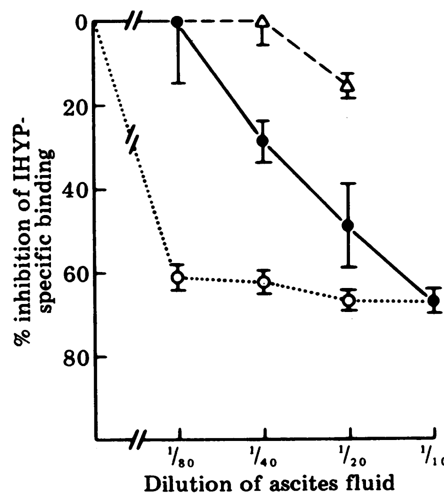

FIG. 2. Competition between IHYP and monoclonal antibody FV-104 for the adrenergic ligand binding site of calf lung β-adrenergic receptors. Partially purified calf lung membranes (1.6 fmol of β-adrenergic receptors) were preincubated with serial dilutions of monoclonal antibody FV-104, and IHYP-specific binding to β-adrenergic receptors was determined with 300 pM IHYP (twice K value, open triangles), 150 pM IHYP (equal to K value, solid circles), and 75 pM IHYP (half the K value, open circles) as described in the legend of Fig. 1. The data are shown as mean ± SEM of triplicate determinations from three separate experiments.

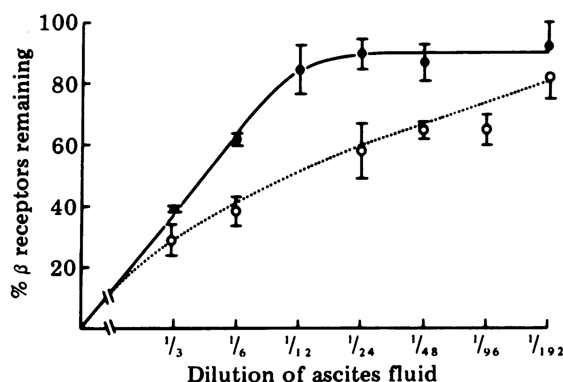

FIG. 3. Immunoprecipitation of partially purified turkey erythrocyte β-adrenergic receptors by FV-103 and FV-104 monoclonal antibodies. Partially purified turkey erythrocyte β-adrenergic receptors (2.1 fmol) were incubated with serial 1:2 dilutions of ascites fluid (in 20 mM $NaPO_4$/2 mM $MgSO_4$, pH 7.4) containing FV-103 (open circles) or FV-104 (solid circles) monoclonal antibodies for 18 hr at 4°C in a final volume of 0.1 ml. Precipitation of β receptor monoclonal antibody complexes was accomplished by incubating samples with 100 μg of rabbit anti-mouse IgG for 4 hr at 4°C. The immune complexes were centrifuged at 12,000 × g for 5 min and the concentration of β-adrenergic receptors present in the supernates was assessed. Aliquots of supernate were incubated with 60 pM IHYP in the presence or absence of 10 μM (l)-propranolol for 30 min at 30°C followed by precipitation of sample protein with 15% polyethylene glycol (3). Control samples contained 2.0 fmol of partially purified turkey erythrocyte β-adrenergic receptors and no ascites fluid. The values shown are mean ± SEM of duplicate determinations from two separate experiments.

Monoclonal antibody FV-104 was coupled to Sepharose 4B and shown to specifically adsorb turkey erythrocyte β receptors. Specificity of this receptor adsorbtion was demonstrated by comparing β receptor binding to monoclonal antibody FV-104 columns to the binding to control columns composed of purified mouse IgG coupled to Sepharose 4B. β receptor binding to control columns was minimal (<20%), whereas >95% of applied receptor was retained on FV-104 columns. Receptor occupation by propranolol decreased the specific receptor binding to the monoclonal affinity column without affecting nonspecific receptor binding to control columns (not shown).

When ^{131}I-labeled partially purified β receptors were eluted from immunoaffinity columns with $NaDodSO_4$ and mercaptoethanol and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis, three major components were identified at 70,000, 31,000, and 22,000 daltons (Fig. 4). To increase the specificity of immunoaffinity isolation of turkey erythrocyte β-receptors, 10 μM l-propranolol was used for elution of β receptors from monoclonal antibody FV-104-Sepharose columns. The eluate, which was labeled with [^{125}I]iodine prior to $NaDodSO_4$/polyacrylamide gel electrophoresis, contained only a single protein with a molecular weight of approximately 70,000.

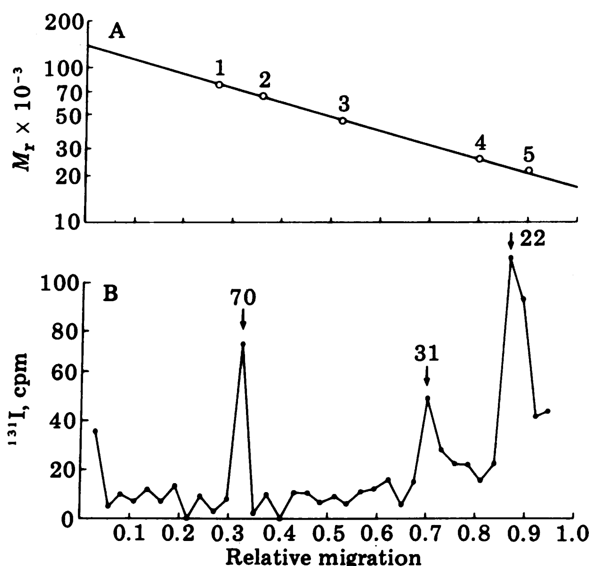

FIG. 4. Monoclonal antibody affinity purification of turkey erythrocyte β-adrenergic receptors. Turkey erythrocyte ghosts were surface labeled with [^{131}I]iodine. β receptors were solubilized from membranes with 0.5% digitonin and purified by preparative isoelectric focusing (13) to a specific activity of 1.67 pmol of receptor per mg of protein. Partially purified receptors (2 ml) were incubated with FV-104 monoclonal antibody-Sepharose 4B (2.5 ml) for 2 hr at 30°C. The Sepharose beads were washed in a 10-ml column with 5 ml of phosphate buffer (pH 7.4). β receptors were eluted from the antibody affinity column with 0.5 ml of buffer containing 1% $NaDodSO_4$, 2.5% mercaptoethanol, and 5% glycerol (17), and samples were incubated at 100°C for 5 min. Column eluates were analyzed on 10% $NaDodSO_4$/polyacrylamide gels (18) which were sliced and assayed for radioactivity. (A) Molecular weight calibration curve for 10% $NaDodSO_4$/polyacrylamide gels using: 1, lactoperoxidase (77,500); 2, bovine serum albumin (66,000); 3, ovalbumin (46,000); 4, chymotrypsinogen A (25,750); and 5, soybean trypsin inhibitor (21,500). (B) ^{131}I-Labeled monoclonal antibody microaffinity column eluates of turkey erythrocyte β-adrenergic receptors. Sizes marked as $M_r \times 10^{-3}$. ^{131}I marked as cpm × 10^{-3}.

DISCUSSION

α- and β-adrenergic receptors and their subclasses mediate dramatically different and often opposing physiological activities, but they share the ability to bind epinephrine and norepinephrine with considerable affinity. This common denominator between the various classes of adrenergic receptors suggests that some degree of structural homology might exist between these molecules, particularly in the region of the adrenergic ligand binding site. We have previously reported on a number of molecular dissimilarities between pharmacological subclasses of β-adrenergic receptors including differences in physical properties as well as a lack of immunological cross-reactivity between lung and cardiac β receptors (3, 4, 10, 11, 20–22).

In the present study we utilized monoclonal antibodies developed to β receptors as probes of β receptor structure. A major advantage of monoclonal antibodies is their homogeneity which permits the study of receptor homology at the level of single antigenic determinants. Monoclonal antibody FV-104 inhibits IHYP-specific binding to turkey erythrocyte and calf lung, liver, and cardiac β receptors with equal efficacy but does not affect [^{3}H]epinephrine binding to rat liver α receptors (Mahmoud El-Rafai and John Exton, personal communication). These data demonstrate that monoclonal antibody FV-104 specifically competes for a determinant located only in the ligand binding site of β-adrenergic receptors. It is possible that the antigenic determinant recognized by FV-104 antibodies is the peptide sequence in the β-adrenergic ligand binding site responsible for the binding specificity of nonselective β-receptor antagonists.

The extent of receptor homology we find with the FV-104 determinant suggests a common genetic heritage for the β receptor with molecular differences in receptor subclasses (3, 4, 22) possibly arising from gene duplication. Other recent evidence from this laboratory also suggests the existence of β receptor homology with respect to the region of the receptor that is responsible for guanine nucleotide regulatory protein interactions (22, 23). This evidence derives from β receptor recon-

stitution data showing coupling of turkey erythrocyte β receptors with mammalian guanine nucleotide regulatory proteins of different cell types (23).

In addition to locating homologous determinants on receptor subclasses, we have demonstrated that monoclonal antibodies have considerable potential in β receptor purification. We utilized monoclonal antibodies coupled to Sepharose 4B to eliminate problems of sample recovery from large immune complexes obtained with indirect β receptor immunoprecipitation. Samples can be directly eluted from monoclonal antibody columns in $NaDodSO_4$ buffer and applied directly to polyacrylamide gels (Fig. 4). When these procedures are performed with [^{131}I]iodine-labeled turkey erythrocyte β receptors, similar molecular weight bands are obtained with greater resolution than can be obtained with radioactive affinity-labeled β receptors (13). We achieved even greater specificity by eluting the turkey erythrocyte β receptors from monoclonal antibody affinity columns with the nonselective β-adrenergic receptor antagonist propranolol. When this material is iodinated with [^{125}I]iodine, a single peak is obtained on $NaDodSO_4$/polyacrylamide gels with a molecular weight of 70,000. These data indicate that receptor material eluted from the monoclonal antibody affinity column with propranolol is a pure, homogeneous protein. We assume that the 70,000-dalton protein is the intact β_1 receptor because it contains the adrenergic ligand binding site and its molecular weight on $NaDodSO_4$/polyacrylamide gels is similar to the hydrodynamic molecular weight for the turkey erythrocyte β receptor (13). The 31,000- and 22,000-dalton peaks (Fig. 4) could be receptor subunits or proteolytic fragments formed from the 70,000-dalton protein.

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