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Research Article

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An Antiidiotypic Antibody That Recognizes the β-Adrenergic Receptor

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ABSTRACT Antialprenolol rabbit antibodies were fractionated on an acebutolol affinity resin, followed by L-propranolol elution so as to separate a class of binding sites that mimic the β-adrenergic receptor. Allotype-identical rabbits were immunized with this fraction. After 6 mo, antisera exhibited antiidiotypic activity inhibiting [^{3}H]alprenolol binding to the original antibody and to rabbit antiacebutolol antibodies, which had a spectrum of ligand-binding properties identical to the original idiotype. Those antisera demonstrating the most potent antiidiotypic activity also blocked [^{3}H]alprenolol binding to the β-adrenergic receptor of turkey membrane, canine pulmonary membrane, and rat reticulocyte. An idiotype affinity-purified fraction showed similar activity, inhibiting β-receptor binding with a calculated dissociation constant (K_D) of 53 nM. Isoproterenol-mediated adenylate cyclase activity was also inhibited in a competitive manner. The universality of recognition of these antiidiotypic antisera indicate that the three-dimensional structure of a receptor's binding site can be modeled by a subset of an elicited antibody population.

INTRODUCTION

Elicited antibodies to the β-adrenergic antagonist alprenolol display binding characteristics similar to the β-receptor itself (1, 2). The remarkable characteristics of this antialprenolol antibody population include a binding affinity in the nanomolar range and general recognition of β-agonists. Moreover, we have previously shown that by taking advantage of affinity purification procedures in conjunction with ligand-specific elution, it is possible to separate a class of antibody binding sites that demonstrate stereospecific recognition properties (2). We had suggested that this affinity-fractionated antibody population could be used as an antigen for the production of antiidiotypic antibodies that might also interact with a similarly structured binding site within the β-adrenergic receptor itself. Such an approach has already been successfully applied with insulin, a far more complex ligand than alprenolol, in the identification of insulin receptors on cell membranes (3). More recently, Schreiber et al. (4) have also reported on the development of antiidiotypic antibodies that recognize the β-receptor. Important contrasts between our results are evident. The antireceptor activity of our antiserum behaves as a classic competitive antagonist of the receptor in contrast to the findings of this group. Furthermore, we have directly assessed the stoichiometry of the idiotype-antiidiotype interaction in an attempt to quantitate the fraction complementary to the ligand-binding site. These findings more clearly outline the utility of this approach as a general method for raising antibodies directed against the binding site of a cellular receptor.

METHODS

Materials. Acebutolol amine (MB 17, 127) was a kind gift of May and Baker, Ltd. (England), L-propranolol HCl of Ayerst Laboratories, NY, and D,L-alprenolol of Astra Pharmaceutical Products, Inc., Framingham, MA. (−)-[^{3}H]Dihydroalprenolol, Aquasol-II, and protosol were obtained from New England Nuclear, Boston, MA. Agarose (Sepharose 4B) and Sephadex G-25 were purchased from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ. Whatman GFC filters were obtained from Scientific Products Div., American Hospital Supply Corp., McGaw Park, IL. ^{125}I-NaI (carrier free) was obtained from Amersham Corp., Arlington Heights, IL. DEAE cellulose was purchased from Whatman, Inc., Chemical Separation Div., Clifton, NJ; CNBr from Kodak, Eastman Kodak Co., Rochester, NY, and all organic chemicals from Aldrich Chemical Co., Milwaukee, WI (highest grade possible). Microzone plates (cellulose acetate) were purchased from Beckman Instruments Inc., Fullerton, CA. Silk screen was purchased from Lambert Co., Inc., Boston, MA. Protein A was purchased from Pharmacia. [^{3}H]Prazosin was obtained from Amersham Corp.

Animals were purchased from Charles River Breeding Laboratories, Wilmington, MA.

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TABLE I

*Inhibition of [^{3}H]Alprenolol Binding to Antibodies and Receptors by Antiidiotypic Antibody**

	Maximal binding with preimmune	In presence of antiidiotype	Inhibition
	dpm		%
Original idiotype (antialprenolol)	10,853±250	488±250	96
Antiacebutolol	6,350±525	1,658±500	74
Turkey erythrocyte	19,643±540	6,195±525	68
Rat reticulocyte	8,248±175	5,410±233	35‡
Canine lung	6,385±283	4,315±190	34

Illustration of the inhibitory effect of antiidiotypic antiserum obtained 24 wk after immunization with antialprenolol antibodies. It is apparent that this antiserum antagonized [^{3}H]alprenolol binding not only to the original idiotype but also to antibodies raised to the ligand acebutolol. 50% inhibition of antibody binding was still seen at a final dilution of 1:60 (data not shown). The antireceptor activity of this antiserum is also apparent in its inhibition of [^{3}H]alprenolol binding to β-receptor of different species and cell types. The assay utilized 25 μl of antiserum in a total volume of 150 μl.

* Specific binding at 4–6 nM [^{3}H]alprenolol.

‡ Purified Ig fraction.

Assays. Antibody binding of ligand was determined by a double antibody precipitation assay as previously described (2). In receptor assays, separation of bound from free ligand was achieved by vacuum filtration onto GFC glass filters as previously described (5).

The adenylate cyclase assay was performed as previously described (6) using the method of Salomon et al. (7) to separate cyclic [^{32}P]AMP. An internal standard, cyclic [^{3}H]AMP, was also used to quantitate recovery in each sample.

Protein concentration was determined by the method of Lowry et al. (8).

Syntheses

Ligand-protein complexes. Acebutolol amine was diazotized as previously described (9) and reacted directly with albumin in 10% Na_2CO_3 buffer. This was then extensively dialyzed against 50 mM Tris buffer, pH 7.4. Alprenolol-albumin was synthesized as previously described (2).

Agarose-ligand resins. 3-Iminobispropylamine was attached to agarose utilizing CNBr activation as described by Cuatrecasas (10) using 60 ml Sepharose 4B, 11.0 g CNBr, and 13.1 g of iminobispropylamine. *p*-Hydroxy-phenylacetic acid (200 μmol) was coupled to the resulting agarose amine (10 ml) using 0.1 M water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl in 20.0 ml H_2O at pH 4.75 (maintained with 2 N HCl) for 1 h. The ninhydrin test showed the reaction to be complete. Acebutolol amine (200 μmol) was then diazotized in 1.0 N HCl and 0.5 M $NaNO_2$ for 10 min, after which it was added to the above resin that had been equilibrated in 10% Na_2CO_3.

Alprenolol-agarose was synthesized as previously described (2). Each of these resins was substituted in the millimolar range as assessed by a direct radioimmunoassay (11).

Immunoadsorbent resins. Immunoglobulin (Ig) was coupled to CNBr-activated Sepharose directly in a 0.1 M $NaHCO_3$ buffer, pH 8.3.

Ig and Fab preparation. The Ig was purified from 2.0 ml serum previously dialyzed into 10 mM phosphate buffer, pH 7.4, and 0.02% NaN_3 by passage over a 20-ml DEAE cellulose column equilibrated in the same buffer. The Ig fraction was then eluted in the same buffer as confirmed by cellulose acetate electrophoresis. Fab was prepared by a modification of the method of Porter (12).

Membrane preparation. Turkey erythrocyte membranes were prepared by a modification of the method of Lad et al. (13) from heparinized turkey blood. A last high-speed centrifugation was performed in 50 mM Tris buffer, pH 7.4. This resulted in a pellet with two distinct layers. The upper, whiter layer was swirled in buffer and removed from nuclear and mitochondrial debris. By phase-contrast microscopy this upper layer consisted principally of enucleated membrane ghosts. By Scatchard analysis these possessed 300–400 fmol β-receptor/mg protein.

Canine lung was obtained from pentobarbital-anesthetized dogs. Hilar vessels, bronchi, and pleura were removed and the lung minced in 1 mM $KHCO_3$, 0.25 M sucrose (5 vol) on ice and then homogenized in a Polytron (Brinkmann Instruments Inc., Westbury, NY) at a setting of 8 × 3 s then at setting 6 × 10 s. The material was then passed through two layers of silk screen and centrifuged at 2,000 rpm in a Sorvall RC-2-centrifuge (DuPont Instruments, Sorvall Biomedical Div., DuPont Co., Newtown, CT) for 10 min. The supernatant was then respun at 15,000 rpm for 20 min and the pellet resuspended to a concentration of 2–3 mg/ml in a 100 mM Tris, 5 mM EGTA, 1 mM $MgCl_2$ buffer, pH 7.4. This preparation contained ~100 fmol/mg membrane protein.

Rat reticulocytes were prepared as described by Wrenn and Homcy (9). These preparations routinely contained ~0.5–1.0 pmol receptor/mg protein.

Liver plasma membranes were prepared by a modification of the method of Song (14) and Ray (15) and contained 200–300 fmol [^{3}H]prazosin binding sites (K_D = 0.5–1.0 nM) per mg membrane protein.

Saturation binding experiments were analyzed by the method of Scatchard (16).

Immunization protocol. Rabbits were immunized with 0.5 mg of the protein-ligand complexes at 3-wk intervals in complete Freund's adjuvant by toepad injection. The protein-ligand complexes were shown by direct radioimmunoassay without preceding hydrolysis to be substituted at a 1:1 molar ratio. High titer (1:1,000-fold dilution at 2 nM [^{3}H]alprenolol) binding was detected after 4 mo of immunization.

To generate an antiidiotypic response, allotype-identical New Zealand rabbits were immunized on a 3–4-wk basis with 0.3 mg of affinity-purified antibody in complete Freund's adjuvant via toepad injection. A battery of antisera for allotype identification was kindly provided by Dr. D. Catty (Department of Immunology, University of Birmingham, Birmingham, U. K.). All of our rabbits were a1,a1,b4,b4.

Iodination. The method of Marchalonis (17) using lactoperoxidase was routinely performed using as little as 25 μg of antibody and 1 mCi of ^{125}I (carrier free). Free iodine was separated on a G-25 Sephadex column (10 ml). Protein A, also iodinated by this method, was kindly provided by Dr. G. Matsueda of this laboratory.

RESULTS

High-affinity antialprenolol antiserum was obtained with an affinity for propranolol in the nanomolar range, as reported previously (2). Those antibodies that recognized features common to the broad range of β-antagonists were purified by affinity fractionation. The antiserum was passed over an acebutolol-affinity resin and then eluted with L-propranolol. Such a procedure results in a class of antibodies that demonstrates stereospecificity in its recognition of β-ligands (2). The eluate was then extensively dialyzed and used for immunization as described.

Of eight rabbits initially immunized with 0.2–0.3 mg of affinity-purified antialprenolol antibody on a 3–4-wk schedule, the antiserum from one rabbit exhibited antiidiotypic activity after 2–3 mo. The assay was performed by incubating 25 μl buffer, preimmune sera, or antiidiotypic antisera with either the original antiligand antibody, or with a membrane preparation when β-receptor binding was analyzed in a total volume of 150 μl. The double-antibody technique was used for the radioimmunoassay system and vacuum filtration for receptor assays to effect separation of bound from free ligand. The activity of an antiserum from a single bleeding at 24 wk after initial immunization demonstrating significant antiidiotypic and antireceptor activity is shown in Table I. Specific binding with preimmune serum in either assay system was typically the same as that found with buffer, although slight degrees of inhibition, never exceeding 10%, were noted at times. The antiidiotypic antiserum almost completely inhibited binding to the original idiotype raised against the ligand alprenolol. Furthermore, [^{3}H]alprenolol binding to antiserum raised to the ligand acebutolol, which possesses a binding affinity and specificity similar to the antialprenolol antiserum, also was inhibited. Finally, inhibition of [^{3}H]alprenolol binding to turkey erythrocyte, rat reticulocyte, and canine pulmonary β-receptors was also observed to range from 35 to 70%. These experiments were performed in the presence of 4 nM [^{3}H]alprenolol.

The time-course of both antiidiotypic activity as determined by inhibition of [^{3}H]alprenolol binding to the original idiotype and antireceptor activity in the serum of this particular rabbit were also assessed. Antiidiotypic activity appeared at 10 wk after immunization, prior to antireceptor activity, which peaked at 26 wk. Both responses disappeared rapidly at 32 wk after the initial immunization. To assess the possible binding of the ligand to the antiidiotypic antiserum itself, a variety of control assays were performed. The antiidiotypic antiserum did not bind by itself any of the [^{3}H]alprenolol, whether separation of bound from free ligand was effected by vacuum filtration or double-antibody precipitation (Table II). (Binding to the original antialprenolol antisera is included for comparison.) Following G-25 gel filtration chromatography of antiidiotypic antiserum initially equilibrated with 4 nM [^{3}H]alprenolol, all of the ^{3}H-ligand appeared in the salt volume with essentially no binding to the macromolecular fraction eluting in the void volume. Furthermore, this same antiserum did not inhibit specific [^{3}H]prazosin binding to the α_1-receptor of rat liver plasma membranes. Using a final concentration of 1 nM [^{3}H]prazosin and 75 μg of liver plasma membranes, 9.2 fmol of specific binding were detected in the presence of buffer, 8.5 fmol with preimmune sera, and 9.0 fmol in the presence of 25 μl of antiidiotypic antisera.

TABLE II

Assessment of Ligand Binding by Antiidiotypic Antiserum

	[^{3}H]Alprenolol (4 nM)	In the presence of (10 μM) (−)-propranolol	Specific binding
		dpm	
RIA by double-antibody technique			
Antiidiotype (50 μl)			
7/9	150	88	63
7/22	165	140	25
Idiotype (50 μl)	300,000	45,000	255,000
GFC filtration			
Antiidiotype 50 μl			
7/22	363	250	113
Preimmune 50 μl	360	278	83

Direct binding of ligand by antiidiotypic antiserum assessed both by double-antibody precipitation and by filtration onto GFC glass fiber filters. Essentially no binding is detected, particularly when contrasted with that obtained with the original idiotype (antialprenolol antibodies). RIA, radioimmunoassay.

The antireceptor binding characteristics of this antiidiotypic antiserum were also assessed by saturation binding analysis using turkey erythrocyte membranes. At each point, total and nonspecific binding were determined in the presence of buffer, preimmune, or antiidiotypic antiserum in the same assay. As seen in Fig. 1A, the affinity constant and total receptor number are identical in the presence of preimmune sera or buffer, but the binding affinity is lower, approximately fourfold to 22 nM in the presence of antiidiotypic antiserum. In that receptor density appeared to be little changed, the antiserum behaved as a competitive inhibitor of receptor binding. Fig. 1B demonstrates the lack of this inhibitory activity in antiserum from a later bleeding where buffer, preimmune,

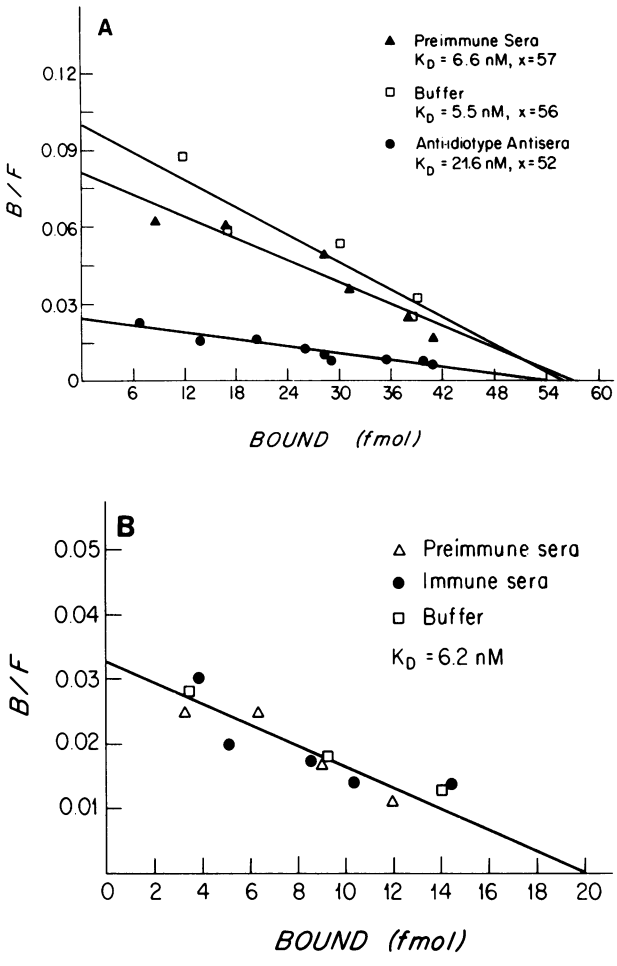

FIGURE 1 Effect of antiidiotypic antiserum on β-receptor function as assessed by Scatchard analysis. Turkey erythrocyte membranes were incubated with increasing concentrations of (−)-[^{3}H]alprenolol, 1–15 nM in the presence of buffer or preimmune serum or 1–45 nM in the presence of antiidiotypic antiserum in a total volume of 150 μl. Total and nonspecific binding (determined in the presence of 1 μM D, L-propranolol) were assessed for each of these at each concentration of (−)-[^{3}H]alprenolol. All points were performed in triplicate. Similar results were obtained in three separate experiments. Fig. 1A shows the effects of antiidiotypic antiserum in a bleed obtained ~24 wk after immunization. Fig. 1B is the same experiment performed using a later bleed (36 wk) when antiidiotypic activity could no longer be detected.

and antiidiotypic curves fall on a line of identity with a receptor affinity of 6.2 nM.

The effects of the DEAE-purified Ig fraction of this antiidiotypic antiserum were also investigated both on receptor binding and isoproterenol-mediated adenylate cyclase stimulation. A competitive inhibition analysis in which increasing concentrations of purified Ig were used to compete with 2 nM [^{3}H]alprenolol for binding to β-receptors in a purified turkey erythrocyte membrane preparation is shown in Fig. 2. Normal rabbit immunoglobulin at the highest concentration had no effect. The effects of this fraction on isoproterenol-mediated adenylate cyclase stimulation in this same membrane preparation (Fig. 2) were studied at two different concentrations of isoproterenol, 0.5 μM, which is the apparent K_D for cyclase stimulation in the turkey erythrocyte, and at a 200-fold higher concentration. At the lower isoproterenol concentration, significant inhibition occurs with most of this effect disappearing at the higher-saturating isoproterenol concentration. This finding is most consistent with competitive inhibition although the slight inhibition seen at 0.1 mM isoproterenol suggests a possible noncompetitive component as well.

The antiidiotypic titer in this rabbit antiserum was also assessed by a direct binding assay. A solid-phase technique was used in which affinity-purified antialprenolol Fab fragments were adsorbed to plastic wells. Antiidiotypic serum was then added at various dilutions and binding to the Fab fragment was detected by the use of iodinated protein-A, which should bind only to antiidiotypic antibodies adsorbed onto the Fab fragments. This technique provided a better measure of the antiidiotypic titer in that detection of binding was via a direct assay that did not depend on the inhibition of high-affinity ligand binding. As shown in Fig. 3, this binding can be demonstrated at a 1:1,000-fold dilution of antiserum, which is clearly higher than that observed for antiidiotypic activity as detected by inhibition of [^{3}H]alprenolol binding to the original antiligand antibody, or by inhibition of β-receptor binding (cf. Table I). Therefore, that fraction of this antiidiotypic response that was hapten inhibitable was also determined by this direct binding assay. Approximately 10% of the total binding at a 1:100-fold dilution of antiidiotypic antiserum is inhibited in the presence of a 1-μM concentration of propranolol. To demonstrate that this inhibition was specific, the antiidiotypic antiserum was purified on an idiotypic affinity resin. Antialprenolol antibodies were first immobilized on agarose, and the antiidiotypic fraction was passed over the resin which was then extensively washed with 100 mM Tris buffer. The antiidiotypic antibodies were eluted with propranolol (1 mM) to obtain a purified subfraction that would compete with the ligand, propranolol, for binding to the original antiligand antibody. This purified antiidiotype was then itself iodinated and used in a solid-phase assay system. Plastic wells were first coated with purified antialprenolol antibodies, and then the iodinated, purified antiidiotypic antibodies were added in the presence or absence of 1 μM propranolol. Inhibition by the ligand, propranolol, approached 80–90%, in contrast

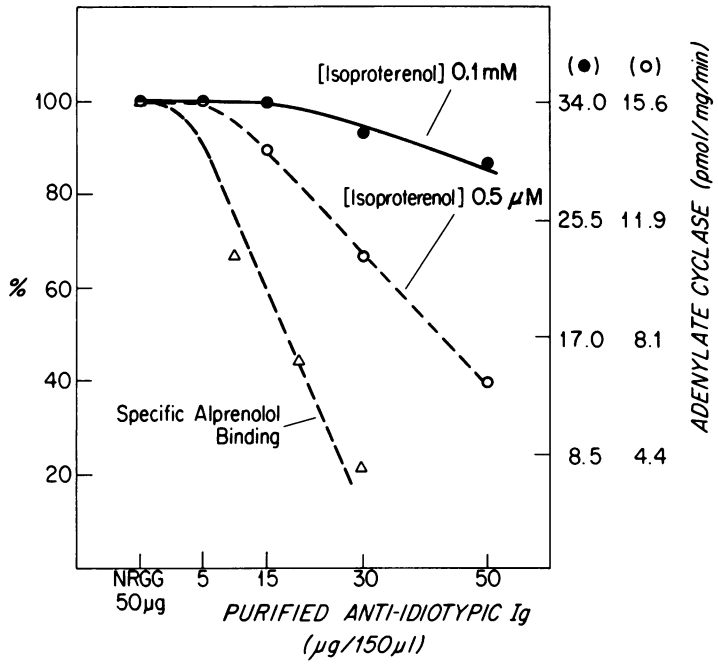

FIGURE 2 The effects of DEAE-purified Ig fraction on (−)-[^{3}H]alprenolol (2 nM) binding to turkey erythrocyte membranes and on isoproterenol-mediated adenylate cyclase stimulation were assessed. The Ig fraction was purified on DEAE-cellulose as described in Methods. The Ig fraction was then dialyzed into 100 mM Tris, pH 7.4. The ability of the antiidiotypic Ig fraction to inhibit adenylate cyclase stimulation in the turkey erythrocyte membrane was tested at isoproterenol concentrations of 0.5 μM and 0.1 mM in the presence of 0.1 mM GTP. In contrast to the effects seen at the nonsaturating isoproterenol concentration of 0.5 μM, only a slight amount of inhibition is seen at the highest Ig concentrations in the presence of 0.1 mM isoproterenol. NRGG, normal rabbit gamma globulin.

to the smaller degree of inhibition seen with a 1:100 dilution of crude antiserum. The converse of this experiment was also performed; that is, plastic wells were first coated with the purified antiidiotypic fraction, and then iodinated, purified antialprenolol antibodies were added. Inhibition of the interaction between these two species by the ligand propranolol approached 90%, demonstrating its specificity. These idiotype-purified antiidiotypic antibodies were then assessed in direct competitive inhibition studies with [^{3}H]alprenolol for binding to the β-adrenergic receptor. 50% inhibition of (−)-[^{3}H]dihydroalprenolol binding (6 nM) to turkey erythrocyte membranes was obtained with an antibody concentration of 0.020 mg/ml. The K_D of this fraction was calculated to be 53 nM by the method of Cheng and Prusoff (18).

DISCUSSION

The impetus for these studies arose from the observation that elicited antiserum to the β-ligand alprenolol possessed a remarkable similarity in its binding properties to the β-adrenergic receptor (2). The immune response produced a class of antibody binding sites that could be easily fractionated by affinity chromatography (2). Based on their relative specificity and affinity, these possessed a high degree of homology to the binding site of the β-receptor. In that antiidiotypic antibodies directed against these binding sites could be generated, it was certainly reasonable to expect that a subset of these might recognize a homologous structure within the β-receptor itself. That such a class of antibodies did exist was suggested by the observation that [^{3}H]alprenolol binding to antibodies raised to a different β-ligand, acebutolol, was significantly inhibited by antiidiotypic antisera raised to affinity-fractionated antialprenolol antibodies. Again, the antiacebutolol antisera had demonstrated binding properties similar to the antialprenolol antisera and to the β-adrenergic receptor itself. Moreover, we have also made the observation that antibodies generated to a third β-ligand, nadolol, possess almost identical binding properties[1] suggesting that the immune response to this

[1] Homcy, C. J. Unpublished observations.

type of structure is restricted in terms of its recognition properties and, furthermore, that these features duplicate those found in the naturally occurring binding site within the β-adrenergic receptor.

Sege and Peterson (3) reported the successful application of this general approach for developing antiidiotypic antibodies to the insulin receptor. However, high antiidiotypic antibody concentrations in the range of 2–3 mg/ml were required in their experiments to effect a 50% inhibition of insulin binding to its receptor and even higher concentrations to mimic its biologic effect. Schreiber et al. (4) used a similar approach in raising antibodies that recognized the β-receptor. These authors also used antialprenolol antibodies as the idiotype. However, DEAE purification alone was used to prepare an Ig fraction for their immunization protocol. Their immunogen would therefore have included unrelated antibodies and, in particular, would be expected to contain high titers of antialbumin antibodies in that an albumin-alprenolol complex had been used as the initial antigen. This contrasts with our approach in which an antibody population whose binding properties had first been refined by affinity fractionation was used for immunization. These differences may explain the variance in our results. Our antiidiotype behaved in a manner most consistent with competitive inhibition, determined both by saturation binding analysis and as assessed by isoproterenol-mediated adenylate cyclase activation. This contrasts with the results of Schreiber et al. (4). By Scatchard analysis, their antibody displayed the characteristics of noncompetitive antagonism. In the presence of 5 mg/ml of antiidiotypic Ig, an ~60% decrease in receptor number occurred with no change in affinity. Whether this is secondary to the action of the antibody at a site other than that which actually binds ligand or to its apparent intrinsic agonist activity is not clear. Their antibody fraction also potentiated the action of epinephrine in stimulating adenylate cyclase even at a maximal catecholamine concentration, an

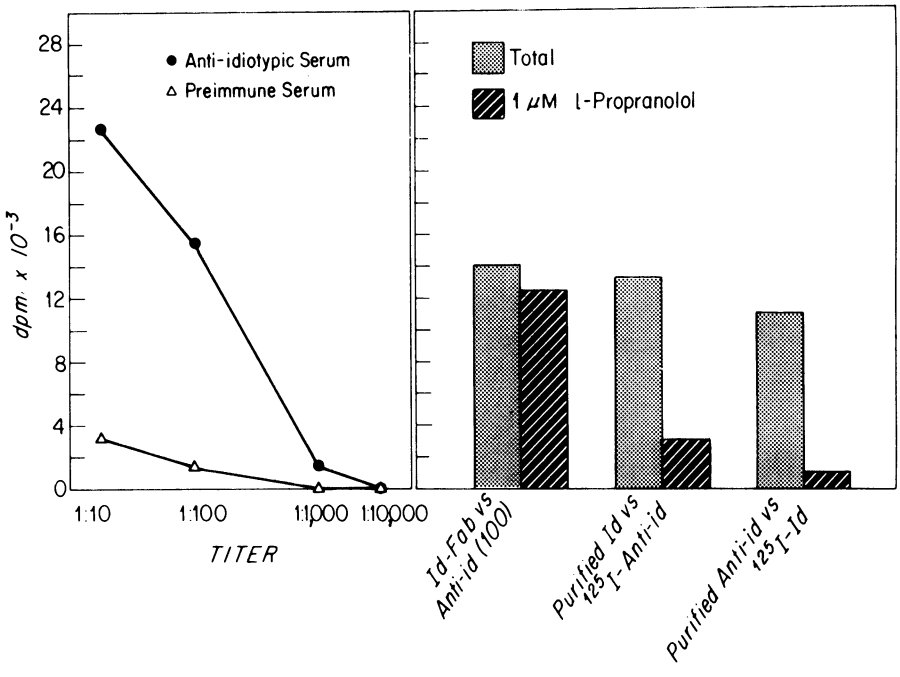

FIGURE 3 Assessment of antiidiotypic activity by a direct binding assay. Left panel: the binding of antiidiotypic antiserum to immobilized antialprenolol Fab fragments was assessed as described in Results. Preimmune serum is also shown. Right panel: measurements of hapten-inhibitable antiidiotypic activity. (A) It can be seen that only 10–15% of antiidiotypic antiserum binding (1:100 dilution) to antialprenolol Fab is inhibited by 1 μM propranolol. (B) Antiidiotypic Ig was first purified by affinity fraction on an immunoadsorbent to which purified anti-alprenolol antibodies were attached. A subfraction was then eluted with L-propranolol (1 mM). Following dialysis this fraction was iodinated and tested for its ability to bind to antialprenolol antibodies previously adsorbed to plastic wells. It is now apparent that 80–90% of this interaction can be inhibited by the ligand, propranolol. (C) The converse of the experiment described above was also performed. Plastic wells were first coated with the affinity-purified antiidiotypic Ig, and then ^{125}I-antialprenolol antibodies were added. A similar degree of inhibition by the ligand is observed.

unusual finding in that epinephrine typically behaves as a full agonist.

By virtue of being identified by three different ligands (the immunogen, alprenolol, the immobilized ligand on the affinity chromatography column, acebutolol, and the eluting ligand, L-propranolol) possessing in common only the propranolamine side chain characteristic of all β-agonists and antagonists, one would expect that the major fraction of the antiidiotype response to our immunogen would be related to the ligand binding site. Fractionation experiments, however, indicate that only a minor fraction of the idiotype-antiidiotype interaction is inhibitable by ligand (Fig. 3). The ligand-inhibitable antiidiotype may be purified by affinity chromatography and provides an extremely interesting reagent for examining the β-receptor site. What, however, is the nature of the major antibody species that recognizes the idiotype, but not its antigen-combining region? A clue is offered in the observation that a variety of β-antagonists seem to elicit similar antibody responses in the rabbit. This situation may be analogous to the observations of Nisonoff (19) in the A/J mouse, where a major fraction of antibodies elicited by an arsonilic acid-protein conjugate share a common idiotype and yet the idiotype-antiidiotype reaction is not entirely inhibitable by arsonilic acid. Recent structural studies of the complementarity regions of the light and heavy chains of these antibodies indicate that there is probably a dissociation between structures that are recognized by the antiidiotype and those involved in antigen binding (20). In fact, monoclonal antibodies have been identified recently that possess this idiotypic determinant, but do not bind arsonilic acid (21). Thus, it is likely that much of the alprenolol antiidiotypic population recognizes determinants within the variable region of the idiotype not relevant either to alprenolol binding by the idiotype nor complementary to the receptor. However, we utilized an idiotype affinity resin and hapten-specific elution to purify a fraction of antiidiotypic antiserum such that a high degree of binding site selectivity was achieved. Compared with unfractionated IgG, an almost 10-fold increase in antireceptor activity was obtained. The K_D of this eluted fraction for the β-receptor was calculated to be 53 nM. It is likely that only a small fraction of even these idiotype affinity-purified antibodies recognize the receptor's binding site, which would suggest that the affinity of this cross-reactive species may be considerably higher than 53 nM. This approaches the affinity of even the most selective β-antagonists presently available.

These studies indicate the suitability of using an elicited antibody response to obtain a population of binding sites whose properties mimic those of a ligand's biologic receptor. In particular, it was our aim to emphasize how a small ligand with a defined number of determinants may be the most appropriate system for such an approach. Suitability arises not only from the simplicity of the ligand's structure, but also from the ability to subfractionate antiligand antibodies by affinity techniques which allows the use of a library of available ligands with similar biologic activity but with different structural determinants. In our particular system, it appears that an additional factor that must be considered is the remarkable similarity in the characteristics of antibodies elicited to any of a variety of β-antagonists. All of these antiligand antibodies were also homologous to the biologic receptor in their binding properties, suggesting that conservation had occurred in the organism's recognition and response to this unique structure. Finally, the antiidiotypic response has been used to raise a population of binding site-directed antibodies. A small fraction of these recognize the three-dimensional structural homology between the antibody's binding site and that of the biologic receptor. However, due to the relatively low titer of these antibodies and the fleeting character of the response, the development of monoclonal antiidiotypic antibodies via somatic-cell hybridization techniques will be necessary before sufficient quantities can be produced to be routinely used as laboratory reagents. The potential specificity afforded by this immunologic approach may allow the development of anti-β-receptor antibodies that possess not only high affinity for the binding site but also a degree of β-subtype selectivity not available with synthetic ligands.

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