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# Autoantibodies and Monoclonal Antibodies in the Purification and Molecular Characterization of Neurotransmitter Receptors

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The combination of immunological advances with membrane receptor research has promoted rapid progress in the molecular characterization of neurotransmitter receptor molecules. We have to date produced monoclonal antibodies to $\beta_1$-, $\beta_2$-, and $\alpha_1$-adrenergic, $D_2$-dopaminergic, and muscarinic receptors. In addition we have discovered that some allergic respiratory disease patients possess circulating autoantibodies to $\beta_2$-adrenergic receptors. These antireceptor antibodies in conjunction with specific receptor affinity reagents have allowed us to isolate, purify, and begin to characterize $\alpha$- and $\beta$-adrenergic, dopaminergic, and muscarinic receptors. For example, immunoprecipitation of turkey erythrocyte $\beta_1$ receptors with monoclonal antibodies yields a single polypeptide $M_r$ 65–70 K. In contrast, purification of $\beta_2$-adrenergic receptors using either autoantibodies or monoclonal antibodies yields a receptor species with a subunit of $M_r$ 55–59 K. Autoantibodies to $\beta_2$ receptors demonstrate a 50–100% homology among $\beta_2$ receptors from humans to rats, whereas monoclonal antibody FV-104 recognizes a determinant in the ligand binding site of all $\beta_1$ and $\beta_2$ receptors tested to date. These data suggest that $\beta_1$- and $\beta_2$-adrenergic receptors may have evolved from a common ancestor, perhaps by gene duplication.

**Key words: neurotransmitter receptors, monoclonal antibodies, muscarinic acetylcholine receptors, receptor purification, receptor subunits, target size analysis, autoantibodies, alpha-adrenergic receptors, beta-adrenergic receptors, dopamine receptors, immunoaffinity chromatography, isoelectric focusing**

At the beginning of this century, Paul Ehrlich defined the concept of cell-surface receptors that recognize and bind complementary ligands [1]. This concept of specific receptors has evolved in a parallel but independent manner in a number of areas including immunology, pharmacology, biochemistry, and endocrinology. Recent advances in unraveling the biochemistry of neurotransmitter receptors are in part a result of the interactions of immunology with receptor biochemistry through the develop-

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ment of specific antireceptor monoclonal antibodies [2–5] and by the discovery of autoantibodies with specificity for a number of cell-surface receptors [6–9].

Our laboratory has developed specific monoclonal antibodies against turkey erythrocyte $\beta_1$-adrenergic receptors, mammalian lung $\beta_2$-adrenergic receptors, rat liver $\alpha_1$-adrenergic receptors, canine brain $D_2$-dopamine receptors, and muscarinic receptors from heart, brain, and intestinal smooth muscle. These anti-receptor monoclonal antibodies in conjunction with autoantibodies against $\beta_2$-adrenergic receptors from asthmatic patients [6,10] have provided new tools for the molecular characterization of these neurotransmitter receptors. These studies in combination with studies utilizing target size analysis and receptor-specific affinity probes have provided substantial information concerning the size and structure of these receptors.

## MATERIALS AND METHODS

### Development of Antireceptor Monoclonal Antibodies

Six-week-old Balb/c mice were immunized with one of the following: partially purified turkey erythrocyte $\beta_1$ receptors as described [11]; rat liver $\alpha_1$-adrenergic receptors purified by preparative isoelectric focusing and gel permeation chromatography; or $D_2$-dopamine receptors purified by preparative isoelectric focusing (see Table I for pI of the various receptor preparations). Booster intraperitoneal injections were administered 4 weeks later and mice were sacrificed 3–4 days subsequent to the booster immunization.

Mouse spleen cells were fused with mouse myeloma SP2/0-Ag14 (SP2) cells according to the method of Gefter et al [12]. SP2 cells do not synthesize immunoglobulin chains [13]. The fusion of spleen cells ($1 \times 10^8$) with myeloma cells ($5 \times 10^7$) in the logarithmic phase of growth was accomplished by addition of 0.2 ml of warm 40% polyethylene glycol 1,000 (PGE 1,000) and centrifugation at 700 rpm for 6 min. The cell suspension was slowly diluted with 12 ml of Dulbecco's 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 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 2× HAT selective medium (hypoxanthine/aminopterin/thymidine) was added to each well. Six to seven half-replacements of the selective medium were performed during the next 1–3 weeks. On days 14–21 after the fusions, supernates from the wells containing hybridomas were assayed for antireceptor antibodies with indirect immunoprecipitation assays (see below). Cells from the positive wells were cloned by limiting dilution and grown as ascites tumors in Balb/c mice primed with Pristane.

### Indirect Immunoprecipitation Assay

Culture medium or ascites fluid were assayed for monoclonal antibodies to each of the respective receptor populations by incubation of samples with partially purified receptors for 18 h at 4°C. Precipitation of receptor-monoclonal antibody complexes was accomplished by addition of rabbit antimouse IgG. Receptor-specific binding was recoverable in the immune precipitates, but the presence of the immune complexes complicated the receptor assays. Therefore, the concentration of receptors remaining in solution after immunoprecipitation was quantitated by labeling receptors as de-

**TABLE I. Molecular Characteristics of Neurotransmitter Receptors**

| Receptor class | $\beta_1$ | $\beta_2$ | $\alpha_1$ | $D_2$ | Brain muscarinic | Heart muscarinic | Smooth-muscle muscarinic |
|---|---|---|---|---|---|---|---|
| Endogenous ligand | Epi-NE | Epi | Epi-NE | DA | ACH | ACH | ACH |
| Hydrodynamic MW | 65 K (dog heart) | 90 K (dog lung and liver) | 83 K (rat liver) | 90 K (dog brain) | 80 K (rat) | — | — |
| Isoelectric point (PI) | 5.5 | 4.2 | 3.9 | 5.0 | 4.0 | 4.0 | 4.0 |
| SDS PAGE MW | (turkey RBC) | (dog lung) | | | (rat, human, dog) | (rat and dog) | (guinea pig ileum) |
| | 65–70 K | 114 K subunit: 55–58 Kdal | 85 K subunit or fragment: 60 K | — | 80 K | 80 K | 80 K |
| | (? subunits, 45, 35, 31 K) | | | | | Tryptic fragments: 65, 52, 42, and 28 K | |
| | | | | | | Apparent structural identify | |
| Radiation inactivation (target size analysis) | 90 K | 115 K | 160 K dog and human brain | 123 K | 80 K | 80 K | 80 K |
| Receptor structure | Monomer? | Dimer $\alpha_2$ or $\alpha\beta$ | Dimer $\alpha_2$ or $\alpha\beta$ | ? | Monomer | Monomer | Monomer |
| Antireceptor antibody | mAb | mAb + Autoantibody | mAb | mAb | mAb | mAb | mAb |
| Cross reactivity | Yes | 50–100% amongst $\beta_2$ receptors only | Specific only for $\alpha_1$ | Specific for dopamine and 5HT receptors | | All muscarinic receptors equal cross reactivity | |

Abbreviations: Epi, epinephrine; NE, norepinephrine; DA, dopamine; ACH, acetylcholine; K, kilodaltons; MW, molecular weight; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; mAb, monoclonal antibody.

scribed below followed by precipitation of labeled soluble receptors with 15% polyethylene glycol [2].

### Receptor Assays

β-Adrenergic receptors were assayed using $^{125}$I-iodohydroxybenzylpindolol (IHYP) as previously described [14]. $\alpha_1$- Adrenergic receptors were assayed with $^3$H-phenoxybenzamine (POB) and prazosin [15,16]; muscarinic receptors were measured using $^3$H-quinuclidinyl benzilate (QNB) and $^3$H-propyl benzilycholine mustard [17]; and $D_2$-dopamine receptors were measured using $^3$H-spiroperidol [18]. All radioactive reagents were from New England Nuclear.

### Radiation Inactivation

Purified membranes were layered 0.5 mm deep and frozen in liquid $N_2$ in open aluminum trays. The membranes were irradiated with a Van de Graff generator producing a 0.5-mA beam of 1.5-MeV electrons. The irradiation chamber was cooled by a stream of liquid $N_2$ to −55°C. Standard proteins and calculations are as described [19].

## RESULTS

The molecular characteristics of the neurotransmitter receptors under study are summarized in Table I. The hydrodynamic molecular weight of each receptor was calculated subsequent to nonionic detergent solubilization from membranes using gel permeation chromatography to determine the Stokes radius of the receptor proteins, and sucrose-density sedimentation studies. Although the molecular weights calculated from these parameters are only approximate, clear differences were apparent in the hydrodynamic properties of the different receptor molecules (Table I).

### Isoelectric Focusing

The single most useful technique in our pursuit of neurotransmitter receptor isolation and molecular characterization has been preparative isoelectric focusing. Figure 1 illustrates results of isoelectric focusing experiments with a number of neurotransmitter receptors. As is not surprising for integral membrane proteins, the receptors are all acidic proteins with pI values ranging from 3.9 to 5.5. In most cases, preparative isoelectric focusing provided at least a 50-fold purification of the receptors with usually 100% recovery of material. In most cases, the isoelectric focusing–

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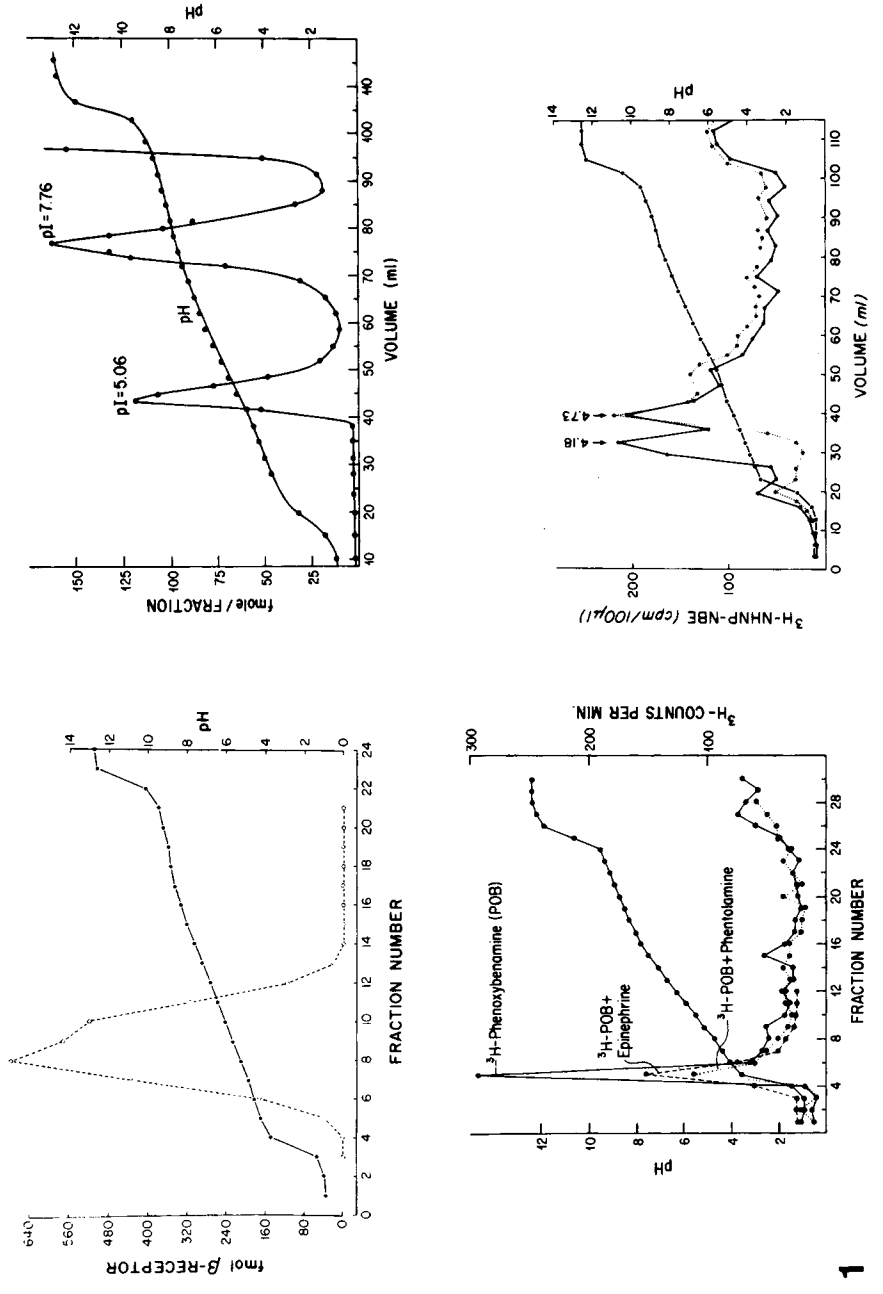
Fig. 1. Preparative isoelectric focusing of solubilized neurotransmitter receptors. Detergent-solubilized receptors were electrofocused for 16 h in a 100-ml column at 15 W constant power. Ampholines (LKB) were included in a linear sucrose stability gradient to develop a pH range of 3–10. Figure in top left is of the turkey erythrocyte $\beta_1$-adrenergic receptor, solublized with 0.5% digitonin as described [2,11]. The fractions were assayed for soluble β-receptor-specific binding as described [2]. The isoelectric point averages 5.5 from over 30 runs. Figure in top right is the $D_2$-dopamine receptor solubilized from canine striatal membranes with digitonin and assayed for $^3$H-spiperone specific binding as described [18,20]. Two peaks appear, pI 5.06 and 7.76; however, the 7.76 peak was found to be associated with detergent micelles whereas the 5.06 peak represents receptor monomers. Lower left and lower right are of rat liver $\alpha_1$-adrenergic and calf lung $\beta_2$-adrenergic receptors respectively, both radiolabeled with covalent affinity reagents as described [15,16,27]. The column isoelectric focusing has proved to be a highly reproducible technique in the isolation and characterization of neurotransmitter receptors.


pI=7.76
pI=5.06
pH
VOLUME (ml)
fmole / FRACTION
4.73
4.18
VOLUME (ml)
$^3$H-NHNP-NBE (cpm/100µl)
$^3$H-Phenoxybenamine (POB)
$^3$H-POB+ Epinephrine
$^3$H-POB+Phentolamine
FRACTION NUMBER
$^3$H- COUNTS PER MIN.
FRACTION NUMBER
fmol β-RECEPTOR

1

purified receptors were the antigen with which mice were immunized for monoclonal antibody production.

## Antireceptor Monoclonal Antibodies

Spleen-cell fusions with mouse myeloma SP2/0-Ag14 (SP2) cells have varied tremendously in fusion efficiency, generating hybridomas in only eight out of 196 wells to 300 out of 300 wells. The most completely characterized monoclonal antibodies to date are those with specificity toward β-adrenergic receptors [2,11]. In addition to these, three monoclonal antibodies have been identified with specificity for $D_2$-dopamine receptors [20], five with specificity for muscarinic receptors, and three specific for $\alpha_1$-adrenergic receptors. The presence and specificity of antireceptor monoclonal antibodies were determined by an indirect immunoprecipitation assay [2]. The application of this assay to turkey erythrocyte β receptors is illustrated in Figure 2. Receptor-specific monoclonal antibodies identified with immunoprecipitation are subsequently tested for their potential effects on radioligand binding to the receptors. Results of these types of assays have provided information concerning the nature and location of the epitopes on the receptor molecules recognized by the monoclonal antibodies. For example, analysis of the effect of anti-β-receptor monoclonal antibodies (Fig. 2) on the binding of $^{125}$I-iodohydroxybenzylpindolol (IHYP) to turkey erythrocyte β receptors is shown in Figure 3. One monoclonal antibody (FV-101) which clearly binds to and immunoprecipitates β receptors has no effect on ligand binding [21], whereas antibody FV-104 acts as a direct competitor of adrenergic

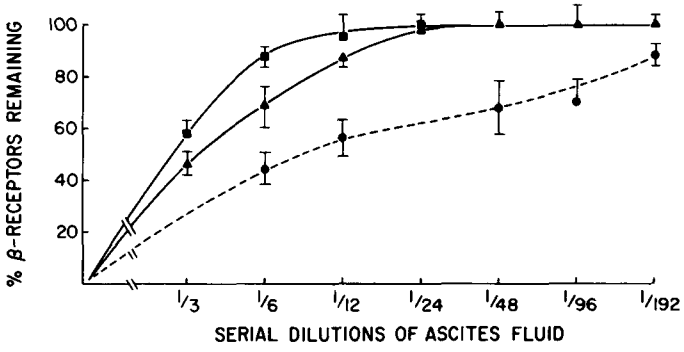



Fig. 2. Indirect immunoprecipitation of soluble turkey erythrocyte β-adrenergic receptors by monoclonal antibodies. β-Adrenergic receptors were solubilized from turkey erythrocyte membranes with 0.5% digitonin as described [2]. Aliquots containing 5 fmol of soluble β receptor were incubated with the indicated serial dilutions of ascites fluid containing monoclonal antibodies to β receptors for 18 h at 4°C. Precipitation of β-receptor-antibody complexes was accomplished by incubation of the samples with 100 μg rabbit antimouse IgG for 4 h at 4°C followed by centrifugation at 12,000g for 5 min. The concentration of β receptors remaining in solution was quantitated by labeling an aliquot of the supernates with IHYP in the presence or absence of 10 μM l-propranolol followed by precipitation of labeled receptors with 15% polyethylene glycol. The values represent the mean ± SEM of triplicate determinations from two separate experiments. The squares indicate the precipitation data obtained with monoclonal antibody FV-101; the circles, with monoclonal antibody FV-103; and the triangles, with monoclonal antibody FV-104.

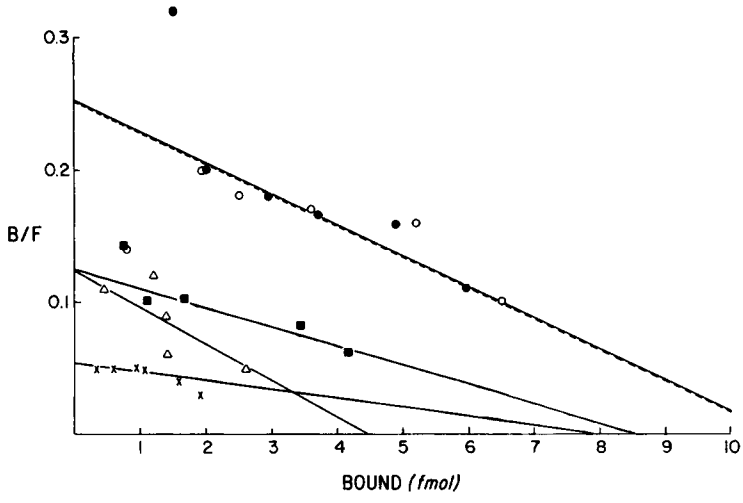



Fig. 3. Scatchard analysis of IHYP saturation isotherms to membrane-bound turkey erythrocyte $\beta$ receptors in the presence of monoclonal antibodies. Turkey erythrocyte membranes containing 10 fmol $\beta$ receptor were preincubated with 1:40 dilution of purified monoclonal antibody, ascites fluid containing antibody, or phosphate-buffered saline containing mouse IgG (30 mg/ml) for 60 min at 30°C. Increasing concentrations of IHYP (25–250 pM) in the presence or absence of 10 $\mu$M l-propranolol were added to the samples and incubated for 30 min at 30°C. The binding reaction was stopped by addition of 1.2 ml of potassium phosphate buffer containing 0.1 mM dl-propranolol followed by filtration through Whatman GF/C glass fiber filters [14]. Scatchard analysis of saturation isotherms obtained in the presence of purified monoclonal antibody FV-104 (■), ascites fluid containing antibody FV-101 (○), FV-103 (△), FV-104 (X), or control mouse IgG (●) are shown. Each point represents the mean of triplicate determinations.

antagonist binding and appears to recognize an epitope within the adrenergic ligand binding site of the receptor [21]. A third antibody, FV-103, has a noncompetitive effect on ligand binding and appears to be a conformationally specific antibody [21].

## Receptor Purification by Monoclonal Antibody Immunoaffinity Chromatography

In addition to being a direct competitor of ligand binding to turkey $\beta_1$ receptors, monoclonal antibody FV-104 has essentially equal cross reactivity with all $\beta_1$- and $\beta_2$-adrenergic receptors studied to date [2]. That the determinant recognized by monoclonal antibody FV-104 is within the ligand-binding site of the receptor and common to both $\beta_1$ and $\beta_2$ receptors provides a unique opportunity with regard to receptor purification [2]. Furthermore, the relatively low affinity of monoclonal antibody FV-104 for the $\beta$ receptor [2] makes this antibody particularly well suited for affinity-purification procedures.

We have capitalized on these properties by binding both $\beta_1$ and $\beta_2$ receptor subclasses to monoclonal antibody FV-104 affinity columns and eluting receptors with low concentrations of the $\beta$-receptor antagonist, l-propranolol [11]. This procedure adds a clear link between ligand-specific affinity chromatography procedures and immunoaffinity chromatography.

The results of turkey erythrocyte $\beta_1$-receptor purification using monoclonal antibody FV-104 are illustrated in Figure 4. Iodination of material eluted from immunoaffinity columns with propranolol reveals a single protein of molecular weight 65–70 K on $NaDodSO_4$-polyacrylamide gels [11]. The eluted protein binds $\beta$-adrenergic ligands and can be reconstituted into acceptor membranes [22], strongly suggesting that the protein species isolated by these techniques is the intact turkey erythrocyte $\beta_1$ receptor.

## Target Size Analysis of Neurotransmitters

To ascertain whether the proteins we have been purifying represent receptor subunits or intact receptors, we undertook a series of studies utilizing radiation inactivation or target size analysis. Radiation inactivation is the only method that can provide the size of a receptor molecule while in the native membrane. This technique has been applied to a number of membrane proteins including the adenylate cyclase system [23], and more recently the nicotinic acetylcholine receptor [24] and insulin receptor [25]. Purified membranes containing the different neurotransmitter receptors under study were subjected to a high-energy electron beam as described [19]. Molecular weights were determined by direct extrapolation from a standard curve of proteins of known molecular weight.

For example, Figure 5 illustrates the loss of $\alpha_1$-adrenergic receptor protein, identified on SDS-polyacrylamide gels, as a result of radiation exposure. These data as well as data from ligand-binding studies indicate that the average size of the $\alpha_1$ receptor in the membrane is 160 K (Table I). In contrast, when target size analysis is applied to muscarinic receptors, the results (Fig. 6) indicate that the muscarinic receptor exists in the membrane as a 80-K unit (Table I).

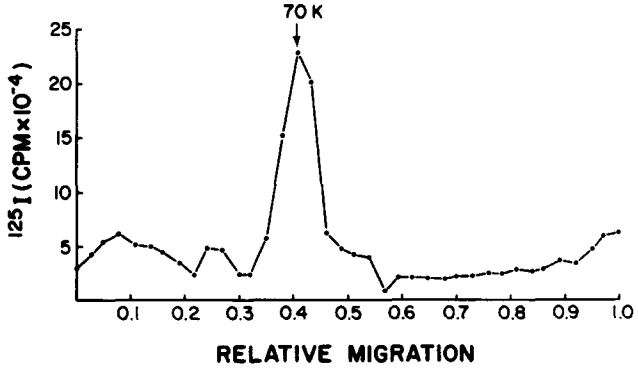



Fig. 4. Ligand-specific elution of turkey erythrocyte $\beta$-adrenergic receptors from immunoaffinity columns. Partially purified turkey erythrocyte $\beta$-adrenergic receptors (600 fmol) were applied to immunoaffinity columns of monoclonal antibody FV-104–Sepharose 4B (5 ml gel). Columns were washed with 10 ml phosphate buffer, and adsorbed $\beta$-adrenergic receptors were eluted with 10 ml of 10 $\mu$M 1-propranolol. The eluate was iodinated with Na$^{125}$I and analyzed on 10% $NaDodSO_4$-polyacrylamide gels which were sliced and counted for radioactivity.

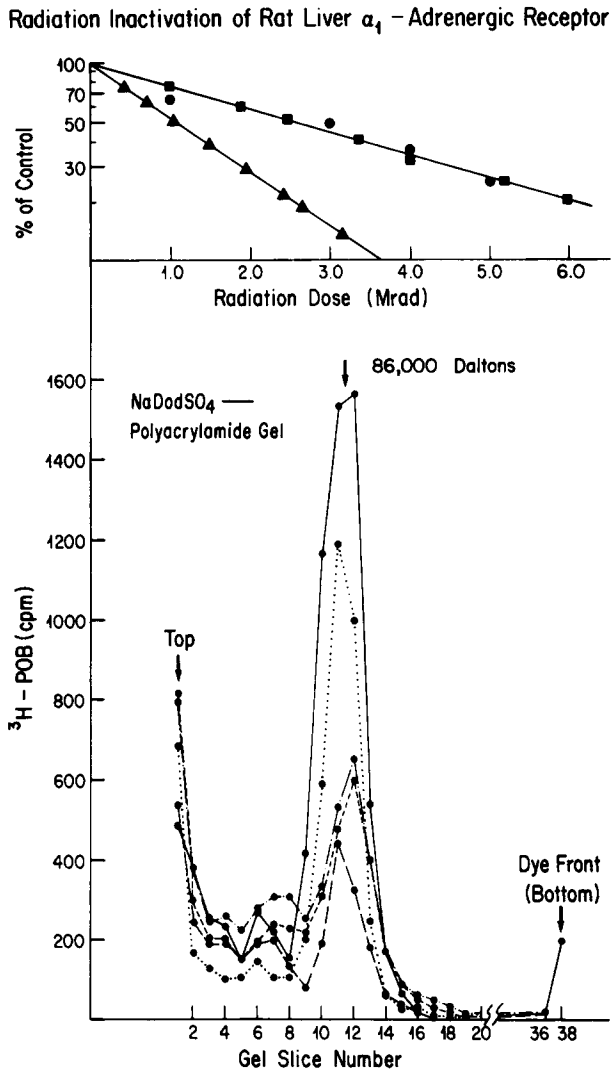



Fig. 5. Radiation inactivation-target size analysis of the rat liver α-adrenergic receptor. Purified rat liver membranes were frozen in thin aluminum trays and subjected to high-energy electron bombardment as described [19]. Samples were thawed and the $\alpha_1$-adrenergic receptors were covalently labeled with $^3$H-phenoxybenzamine, a covalent $\alpha_1$-affinity reagent [15,16]. Radiolabeled α receptors were solubilized with $NaDodSO_4$ (2%) and analyzed by $NaDodSO_4$ polyacrylamide gel electrophoresis as described [16]. The increasing doses of radiation produced a loss of the major $\alpha_1$-receptor subunit $M_r$ 86,000. When the loss or inactivation of the $M_r$ 86,000 subunit is analyzed (top) it is found that the 86,000-dalton unit derived from a membrane complex of $M_r \times 160{,}000$, indicating that the $\alpha_1$ receptor may be a dimer of two 86,000-dalton units. The lower figure illustrates the superimposed $NaDodSO_4$ gel patterns from control membranes (●—●); and membranes subjected to inactivation with 1.0 Mrad (●.....●); 3.0 Mrad (●.—.●); 4.0 Mrad (●----●); and 5.0 Mrad (●- - -●). The upper figure is a semi logarithmic plot of the loss of the 85 kDa $\alpha_1$-receptor monomer (closed circles) from $NaDodSO_4$ gels. Compared on the same plot are the standard enzymes yeast ADH (160 kDa) (closed squares) and β-galactosidase (closed triangles). These data show that the 85 kDa protein derived from a 160 kDa complex in the membrane.

## DISCUSSION: RECEPTOR STRUCTURE

### $\beta_1$-Adrenergic Receptors

The majority of information on $\beta_1$-receptor structure concerns the turkey erythrocyte $\beta_1$ receptor, although hydrodynamic data indicate that canine heart $\beta_1$ receptors have the same hydrodynamic molecular weight (65,000 daltons) as turkey erythrocyte $\beta_1$ receptors [26] (Table I). Our data on the SDS-PAGE molecular weight of the turkey erythrocye $\beta_1$ receptor from monoclonal antibody immunoaffinity chromatography indicate that the receptor is a single polypeptide with a molecular weight of 65–70 K [2,11], which is in good agreement with the hydrodynamic data and also the molecular weight obtained by Strosberg and associates (this volume), who reported finding peptide molecular weights of 70 K and 60 K as well as smaller fragments from immunoprecipitation of turkey erythrocyte $\beta_1$ receptors using anti-idiotypic antibodies.

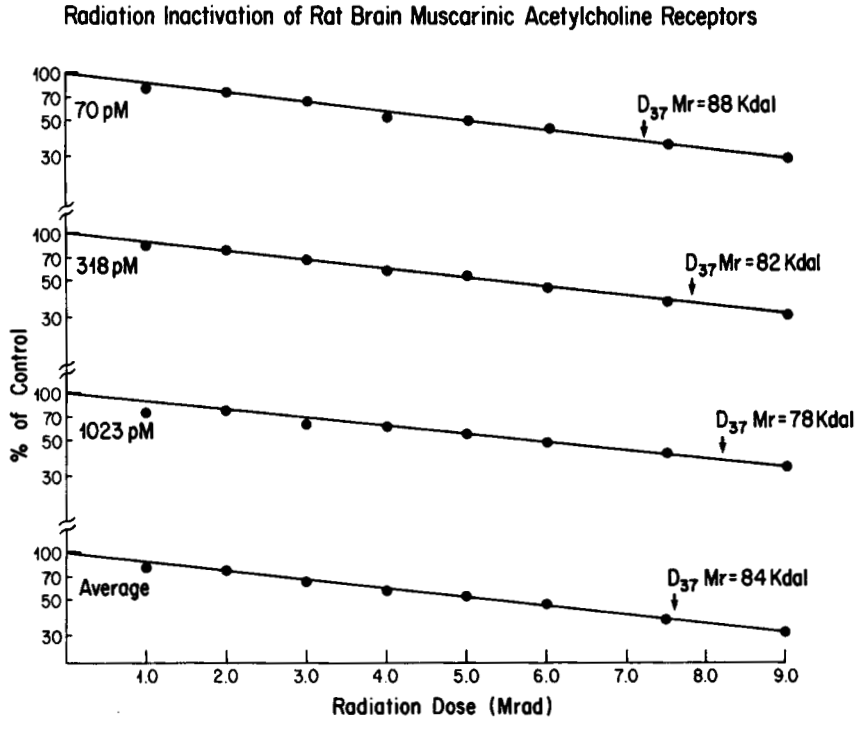



Fig. 6. Radiation inactivation–target size analysis of the rat brain muscarinic acetylcholine receptor. Rat brain membranes were subjected to high energy electron bombardment as described in the legend to Figure 5 and in Methods. Radiation-induced loss of muscarinic receptors was assessed by measuring the loss of $^3$H-QNB specific binding over a wide range of ligand concentrations. The loss was linear over a wide range of radiation doses. The average molecular weight was calculated to be 84,000, nearly identical to the $NaDodSO_4$ molecular weight of 80,000 from a variety of tissues (Table I).

Radiation inactivation analysis of turkey erythrocyte $\beta_1$ receptors originally reported by Rodbell and co-workers [23] (Table I) indicates a molecular weight for this protein in the membrane of 90 K. The error inherent in this type of analysis is on the order of 15% [24]. It is therefore not clear if the 65–70 K protein isolated by immunoaffinity chromatography represents the entire turkey erythrocyte $\beta$ receptor. However, the protein purified with immunoaffinity techniques does retain ligand-binding properties and can reconstitute with the guanine nucleotide regulatory protein of adenylate cyclase [22]. Therefore, the best estimate at this time is that the $\beta_1$ receptor is a monomer and a single polypeptide chain of 65–70 K (Table I). As mentioned in the Results section, the turkey erythrocyte $\beta$ receptor shares at least one determinant with all $\beta_1$ and $\beta_2$ receptors studied to date, indicating that $\beta_1$- and $\beta_2$-adrenergic receptors may have arisen as a result of gene duplication [2].

## $\beta_2$-Adrenergic Receptors

Canine lung $\beta_2$ receptors were found a few years ago to have a primary subunit with a molecular weight of 59 K [27]. This is similar to the value reported for frog erythrocyte $\beta$ receptors [28]. However, from the beginning of the molecular studies on mammalian $\beta_2$ receptors there have been lines of evidence for a species of higher molecular weight [27]. The hydrodynamic molecular weight of $\beta_2$ receptors was calculated to be 90 K with a Stokes radius of 5.8 nm as compared to 4.2 nm for turkey erythrocyte and heart $\beta_1$ receptors [26,27]. Immunoprecipitation of lung $\beta_2$ receptors with either autoantibodies or monoclonal antibodies followed by SDS-polyacrylamide gel electrophoresis generates the basic subunit $M_r$ 55–58 K as well as a protein of $M_r$ 114 K which appears occasionally [27]. Target size analysis has proved very valuable with regard to the $\beta_2$ receptor, as it has revealed a molecular weight of 115 K for the lung $\beta_2$ receptor in the intact membrane (Table I). The evidence therefore indicates a structural size of 90–120 K for the intact $\beta_2$ receptors with a subunit of 50–59 K. Studies with human lung $\beta_2$ receptors purified with monoclonal antibody FV-104 indicate that the intact receptor of 90 K is composed of two disulfide-linked monomers of 47 K [11]. We surmise that the $\beta_2$ receptor is most likely a dimer of two identical subunits, although a structure of two dissimilar subunits cannot be completely eliminated at this point (Table I). Antibody data indicate 50–100% cross reactivity of $\beta_2$ receptors from a number of species and tissues, suggesting that the adrenergic receptors are highly conserved proteins [6].

## $\alpha_1$-Adrenergic Receptors

$\alpha$-Adrenergic receptors bind the same hormone (epinephrine) and neurotransmitter (norepinephrine) as do $\beta$-adrenergic receptors; however, the $\alpha$ receptors often mediate other physiological functions than $\beta$ receptors in the same tissue. The specificity of this diversity appears to rest to a considerable extent in the structure of the receptor protein. Structurally, the $\alpha_1$ receptor differs substantialy from the $\beta$ receptor (Table I). $^3$H-Phenoxybenzamine has been successfully used as a covalent $\alpha_1$-receptor affinity reagent [15,16], and has allowed the isolation and molecular-weight determination of the $\alpha$-receptor protein. A single polypeptide $M_r$ 80–85 K

has been identified on SDS-polyacrylamide gels [16]. The SDS molecular weight is similar to the hydrodynamic molecular weight for the $\alpha_1$-adrenergic receptor (Table I). Radiation inactivation studies (see Fig. 5), however, show that the 85-K protein arises from a complex in the membrane with an average molecular weight of 160 K (Table I). This number would suggest that the $\alpha_1$ receptor is at least a dimer, either of two 85-K subunits or one such subunit which contains the ligand-binding site and a second subunit yet to be identified. Monoclonal antibodies raised against $\alpha_1$ receptors are specific for the $\alpha_1$ receptor, with no apparent cross reactivity with $\beta$-adrenergic or dopamine receptors (Table I). Anti-$\beta$-receptor antibodies to date show no cross reactivity with $\alpha$ receptors [2].

## $D_2$-Dopamine Receptors

While the data are at present more limited on dopamine receptor structure, rapid progress is being made. Target size analysis data indicate a molecular weight of 123 K for the brain $D_2$-dopamine receptor which is similar to its hydrodynamic molecular weight and in the range of the other neurotransmitter receptors [29]. Monoclonal antibodies raised against the canine brain $D_2$ receptor immunoprecipitate the $D_2$ receptor from a number of sources including human brain. One monoclonal antibody made to the $D_2$-dopamine receptor can also immunoprecipitate the 5HT receptor from canine brain. These data together with those given above have prompted the speculation of an adrenergic receptor evolutionary pattern as shown in Figure 7.

## Muscarinic Receptors

Muscarinic receptors isolated from rat brain, human brain, dog brain, rat and dog heart, and guinea pig ileum smooth muscle all have molecular weights of 80 K and appear to be single polypeptides [17] (Table I). We therefore investigated the possibility of structural similarities of muscarinic receptors from these diverse species and tissues. Limited proteolysis studies [17] show that essentially identical tryptic fragments can be generated from each muscarinic receptor (Table I). Monoclonal antibodies generated against the guinea pig ileum muscarinic receptor cross-react equally with all muscarinic receptor proteins studied to date (Table I). Radiation inactivation studies (Fig. 6) have demonstrated that the muscarinic receptor exists as an 80-K single polypeptide in intact membranes. The muscarinic receptor appears to

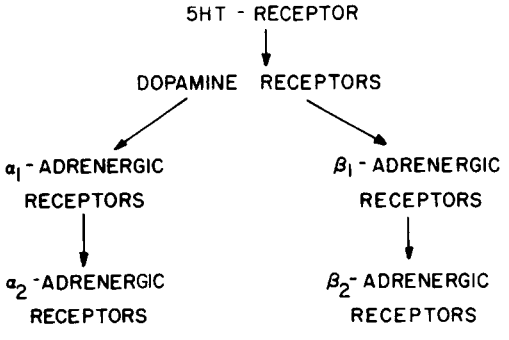



Fig. 7. Evolution of neurotransmitter receptors.

be a monomer of 80 K and, although there may be microheterogeneity between muscarinic receptors from different species and tissues, they appear to be structurally identical at the levels studied to date. Muscarinic receptors are also highly conserved proteins.

There is no apparent structural identity between muscarinic and nicotinic acetylcholine receptors as immunoprecipitation studies with 160 monoclonal antibodies to nicotinic acetylcholine receptors developed by Lindstrom and co-workers (Venter, unpublished) demonstrated no cross reactivity with muscarinic receptors.

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