*Proc. Natl. Acad. Sci. USA*
Vol. 81, pp. 272–276, January 1984
Neurobiology

# Monoclonal antibodies detect the conservation of muscarinic cholinergic receptor structure from *Drosophila* to human brain and detect possible structural homology with $\alpha_1$-adrenergic receptors

(anti-receptor monoclonal antibodies/isoelectric focusing/sodium dodecyl sulfate/polyacrylamide gel electrophoresis/receptor evolution)

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*Communicated by David Harker, September 12, 1983*

**ABSTRACT** **Muscarinic cholinergic receptors isolated from *Drosophila* heads, rat and human brain, dog heart, and monkey ciliary muscle were examined for structural similarities/differences by utilizing isoelectric focusing, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and monoclonal antibody crossreactivity. Muscarinic receptors were affinity labeled with [$^3$H]propylbenzilylcholine mustard and subjected to isoelectric focusing. Muscarinic receptors from each species focused with an isoelectric point of 5.9. The same proteins all migrated with an apparent molecular mass of 80,000 daltons on sodium dodecyl sulfate gels. Six hybridomas secreting monoclonal antibodies specific for muscarinic receptors were developed by using purified rat brain muscarinic receptors as the antigen. The six different monoclonal antibodies immunoprecipitated muscarinic receptors from all tissues and species tested, including human and *Drosophila* brains, with equal efficacy. These data indicate that muscarinic receptors are highly conserved over a considerable evolutionary period. One of the six muscarinic receptor monoclonal antibodies also immunoprecipitated rat liver $\alpha_1$-adrenergic receptors. Furthermore, two out of five monoclonal antibodies raised against $\alpha_1$-receptors immunoprecipitated muscarinic receptors. These data suggest that some degree of structural homology exists between muscarinic cholinergic receptors and $\alpha_1$-adrenergic receptors.**

The muscarinic cholinergic receptor mediates various central nervous system activities as well as the function of the parasympathetic side of the autonomic nervous system, including heart rate attenuation and smooth muscle contraction in the eye, intestine, and airways. This laboratory has investigated the structure of the muscarinic cholinergic receptor and found it to be a monomeric protein with a molecular mass of 80,000 daltons (1). Topology studies indicate that the muscarinic receptor is similar to the nicotinic receptor (2) in that over 50% of the receptor protrudes into the extracellular aqueous space and 14% of the receptor is on the cytoplasmic side of the membrane (1).

In contrast to the adrenergic receptor system, major differences do not appear to exist in the structure of muscarinic cholinergic receptors isolated from vertebrate heart, brain, and smooth muscle (1). It appears as though the muscarinic receptor may be highly conserved, perhaps throughout a large part of the evolutionary process. There is evidence to suggest that atropine-sensitive acetylcholine responses, indicative of the presence of muscarinic receptors, exist throughout much of the phylogenetic tree, in molluscs, annelids, and arthropods (3, 4), including insects, in which muscarinic receptor-specific radioligand binding has been reported (5–7).

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$\alpha_1$-Adrenergic receptor monomers are proteins with a molecular mass of 80,000–85,000 daltons (8–10) with a membrane topology and tryptic digest map similar to those of muscarinic receptors (9, 10). In the present study we describe the production of monoclonal antibodies specific for muscarinic cholinergic and $\alpha_1$-adrenergic receptors and their use in detecting structural similarities between muscarinic cholinergic receptors isolated from a wide variety of tissues and between muscarinic receptors and rat liver $\alpha_1$-adrenergic receptors.

## MATERIALS AND METHODS

[*propyl*-2,3-$^3$H]Propylbenzilylcholine mustard ([$^3$H]PrBCM) (92 Ci/mmol; 1 Ci = 37 GBq), as the hydrochloride, and [$^3$H]phenoxybenzamine ([$^3$H]POB) (15–45 Ci/mmol) were from New England Nuclear. Other materials were as described (1, 8–11).

**Tissues and Membranes.** Rat and human brain membranes were prepared as described (1). Wild-type *Drosophila melanogaster* heads were separated from bodies as described (12) and stored at −80°C prior to use. Heads were homogenized in homogenization buffer, consisting of 5 mM sodium phosphate at pH 7.3, 1% sucrose, 10 mM EDTA, 10 mM EGTA, and 0.02% sodium azide and containing additional protease inhibitors aprotinin (20 kallikrein inhibitor units/ml), bacitracin (100 μg/ml), benzamidine (1 mM), benzethonium chloride (0.1 mM), pepstatin (0.1 μg/ml), phenylmethylsulfonyl fluoride (0.1 mM), leupeptin (10 μg/ml), soybean trypsin inhibitor (10 μg/ml), and iodoacetamide (10 mM). Protease inhibitors were made up individually and added to the homogenization buffer immediately prior to use. Homogenization was in a glass homogenizer with a motor-driven Teflon pestle (30 strokes at 1,700 rpm) continuously cooled to 0°C. Homogenates were centrifuged at 2,000 × *g* for 10 min. The supernate was saved and the pellet was resuspended in fresh homogenization buffer and rehomogenized and recentrifuged as above. This step was repeated three times and the supernates were combined and centrifuged at 37,000 × *g* for 45 min. The supernate was discarded and the upper pink part of the pellet was resuspended in fresh homogenization buffer containing protease inhibitors. Membranes were divided into aliquots, snap frozen in liquid $N_2$, and stored at −80°C prior to use.

Rat liver plasma membranes were prepared as described (10), using the above protease inhibitors. Canine heart membranes were prepared as described (1), and monkey ciliary muscle membranes were provided by J. Polansky (University of California, San Francisco).

**Monoclonal Antibody Production.** Muscarinic receptors were purified by a sequential combination of detergent solubilization, preparative isoelectric focusing, gel permeation

Abbreviations: [$^3$H]PrBCM, [*propyl*-2,3-$^3$H]propylbenzilylcholine mustard; [$^3$H]POB, [$^3$H]phenoxybenzamine.

chromatography, and preparative $NaDodSO_4$/polyacrylamide gel electrophoresis on an LKB Uniphor as described (13). Six-week-old BALB/c mice received intraperitoneal injections of purified rat brain muscarinic receptor emulsified in an equal volume of Freund's complete adjuvant (for muscarinic receptor antibodies) or rat liver membranes (for $\alpha_1$-receptor antibodies). Booster injections were given 4 weeks later and mice were sacrificed on the fourth subsequent day. The mouse spleen cells were fused with mouse myeloma SP2/0 Ag14 (SP2) cells as described by Fraser and Venter (11) and Fraser and Lindstrom (14). Cells from positive cultures were cloned by limiting dilution and grown as ascites tumors in BALB/c mice primed with pristane (11, 14).

**Screening Assays.** Spent culture medium or ascites fluid was incubated with purified or partially purified affinity-labeled muscarinic receptors or affinity-labeled $\alpha_1$-adrenergic receptors at 4°C for 18 hr. Antiserum to mouse IgG was added to the reaction mixture, and incubations were continued for an additional 4–6 hr, and then terminated by micro centrifuge centrifugation (5 min, 4°C).

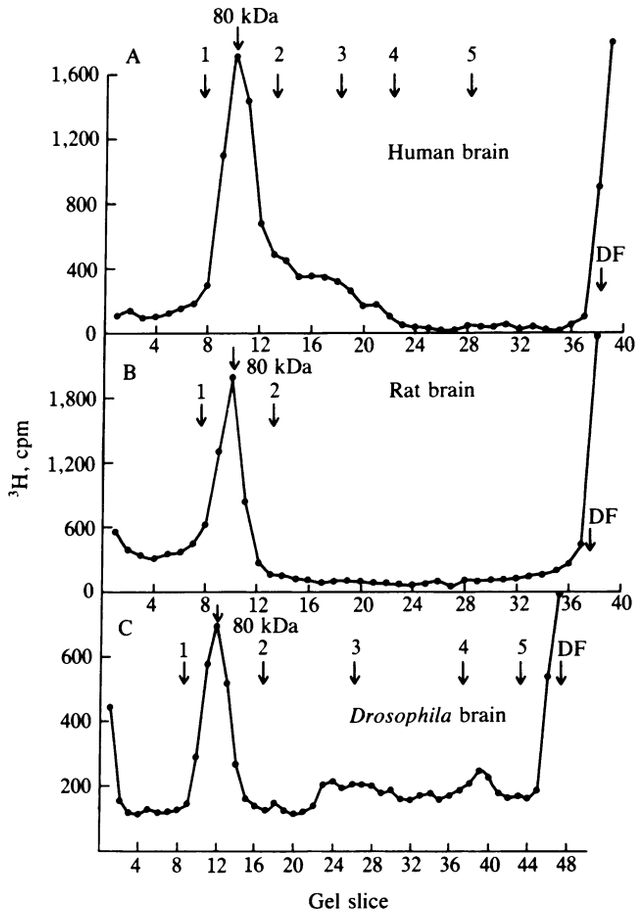


FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis molecular mass of affinity-labeled muscarinic cholinergic receptors isolated from human, rat, and *Drosophila* brain. Membranes were purified from the indicated tissues and affinity labeled with 1 nM [$^3$H]PrBCM. [$^3$H]PrBCM–muscarinic receptor complexes were solubilized with 2% $NaDodSO_4$ and analyzed on 10% $NaDodSO_4$ gels. Samples were treated at 100°C for 5 min in the presence of 5% (vol/vol) 2-mercaptoethanol. Gels were cut into 2.0-mm slices and their radioactivities were measured in a scintillation counter after an overnight incubation in Econofluor (New England Nuclear) containing 3% Protosol (New England Nuclear). The data in each panel are representative of data from at least three experiments. $NaDodSO_4$ gels from affinity-labeling experiments in the presence of 1 $\mu$M atropine show radioactivity only at the dye front (DF). Standard proteins were analyzed on each gel. Protein standards are as follows: 1, phosphorylase b (94,000 daltons); 2, albumin (67,000 daltons); 3, ovalbumin (43,000 daltons); 4, carbonic anhydrase (30,000 daltons); and 5, trypsin inhibitor (20,000 daltons) (Pharmacia electrophoresis calibration kit). kDa, Kilodaltons.

**Isoelectric Focusing of Receptors.** Isoelectric focusing was performed as described (15) in a 110-ml LKB column at 4°C, using 1% Ampholine (LKB), pH range 3–10, for 16 hr under constant power (15 W) with an end point of 1,600 V and 3–4 mA. The pH of the column fractions was determined within 30 min of fractionation at 4°C.

**$NaDodSO_4$/Polyacrylamide Gel Electrophoresis.** $NaDodSO_4$/polyacrylamide gel electrophoresis was performed as described (1), using the method of Laemmli (16).

**Receptor Affinity Labeling.** Muscarinic cholinergic receptors were covalent affinity labeled with [$^3$H]PrBCM at 1 nM in the presence or absence of 1 $\mu$M atropine as described (1).

Rat liver $\alpha_1$-adrenergic receptors were covalently affinity labeled with [$^3$H]POB at 1 nM in the presence or absence of 1 $\mu$M prazosin as described (8, 10).

## RESULTS

**$NaDodSO_4$/Polyacrylamide Gel Electrophoretic Analysis of Muscarinic Receptor Structure.** When membranes isolated from human brain, rat brain, and *Drosophila* heads are affinity labeled with [$^3$H]PrBCM, solubilized with $NaDodSO_4$, and analyzed on $NaDodSO_4$/polyacrylamide gels, a single specifically labeled peak (presence and absence of 1 $\mu$M atropine) appears with an apparent molecular mass of 80,000 daltons (Fig. 1). These data demonstrate that the *Drosophila* muscarinic cholinergic receptor has the same apparent molecular mass as the human brain receptor and are consistent with data obtained from a variety of techniques and tissues (Table 1) which indicate that the muscarinic cholinergic receptor is a monomeric protein of 80,000 daltons in tissues studied to date.

Table 1. Muscarinic cholinergic receptor structure

| Tissue | Molecular mass, daltons | Ref. | Isoelectric point, pH |
|---|---|---|---|
| Human | | | |
| Brain | 78,000 ± 1,200 (PAGE) | 1 | 5.9 |
| | 82,000 (Target size) | 1 | |
| | 80,000 (PAGE) | — | |
| Monkey | | | |
| Ciliary muscle | 80,000 (PAGE) | — | 5.9 |
| Canine | | | |
| Brain | 82,000 ± 1,800 (PAGE) | 1 | |
| Heart | 81,000 ± 3,000 (PAGE) | 1 | 5.9 |
| Rat | | | |
| Brain | 82,000 (Target size) | 1 | 5.9 |
| | 86,000 (Hydrodynamic) | 17 | |
| | 80,000 ± 2,000 (PAGE) | 1 | |
| | 83,200 ± 2,500 (PAGE) | 18 | |
| Heart | 78,000 ± 1,800 (PAGE) | 18 | — |
| | 78,000 (Target size) | 1 | — |
| Guinea pig | | | |
| Ileum smooth muscle | 79,000 ± 4,200 (PAGE) | 1 | |
| | 77,600 ± 2,000 (PAGE) | 18 | |
| Brain | 83,200 ± 6,000 (PAGE) | 18 | — |
| Frog | | | |
| Brain | 80,000 (PAGE) | — | — |
| *Drosophila* | | | |
| Head | 80,000 (PAGE) | — | 5.9 |

Data are from the present study unless otherwise indicated by a reference number. Methods for molecular mass determination were $NaDodSO_4$/polyacrylamide gel electrophoresis (PAGE), radiation inactivation/target size analysis (Target size), or hydrodynamic analysis. Isoelectric points were determined as described for Fig. 2.

**Isoelectric Focusing of Muscarinic Receptors.** To further examine the similarities and differences in muscarinic receptors from diverse species and tissues, the affinity-labeled detergent-solubilized proteins were characterized by isoelectric focusing (Fig. 2 and Table 1). The isoelectric point (pI) of a protein is a parameter that can indicate minor differences in protein structure. The isoelectric focusing profiles of human and rat brain and *Drosophila* head muscarinic receptors (Fig. 2) illustrate that muscarinic receptors from these tissues have essentially identical isoelectric points (pI 5.9), further supporting the notion of structural similarities between these receptors. Cardiac and smooth muscle muscarinic receptors also have a pI of 5.9 (Table 1). There are some effects of $NaDodSO_4$ solubilization on the pI of the muscarinic receptor, because digitonin solubilization results in a pI of 4.2 for the muscarinic receptor from each tissue (unpublished data). This difference could be due to residual $NaDodSO_4$ or to a more complete stripping of lipid by the $NaDodSO_4$ solubilization.

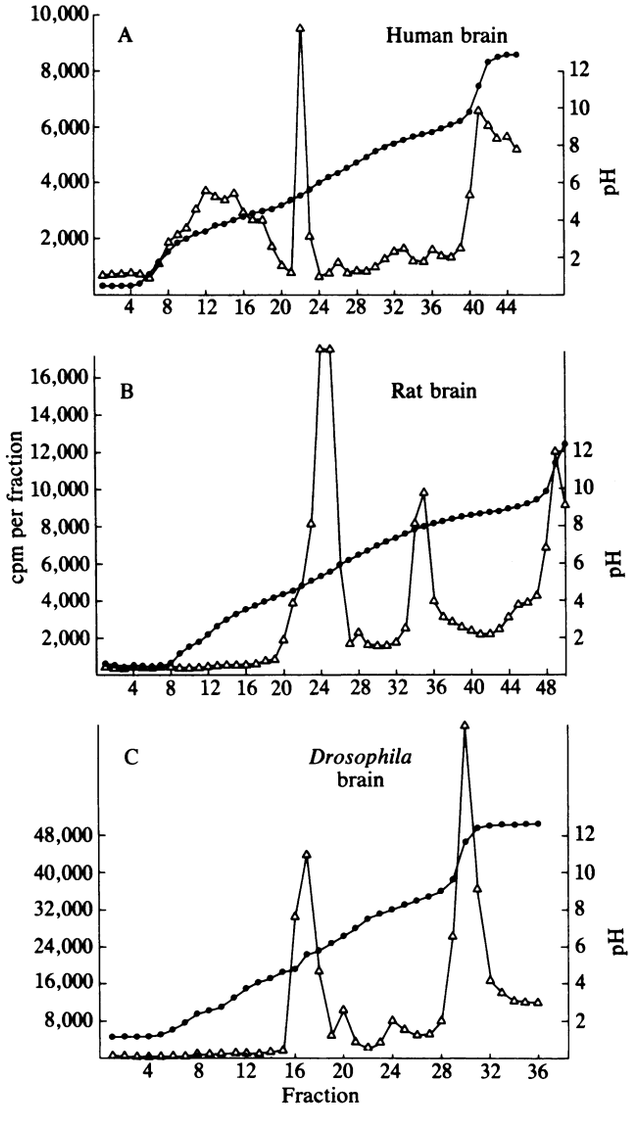


FIG. 2. Isoelectric focusing of human, rat, and *Drosophila* brain muscarinic cholinergic receptors. Affinity-labeled muscarinic receptors ([$^3$H]PrBCM) were solubilized from the indicated tissues with 2% $NaDodSO_4$ and applied to a 110-ml isoelectric focusing column (LKB) containing Triton X-100 (0.1%) and 1% Ampholine (LKB), pH range 3–10, stabilized in a 0–50% sucrose density gradient at 4°C. Data in each panel are representative of data from at least three experiments. Receptors that were affinity labeled in the presence of 1 $\mu$M atropine and analyzed by isoelectric focusing display no radioactivity at pI 5.9, regardless of which tissue was labeled. The isoelectric point (pI) of 5.9 was consistently obtained from human, rat, and *Drosophila* brain muscarinic receptors. Radioactive peaks at pI 11–12 represent free ligand. ●, pH gradient; △, cpm per fraction of [$^3$H]PrBCM. There are no major protein bands that migrate with a pI of 5.9.

**Production and Characterization of Anti-Muscarinic Receptor Monoclonal Antibodies.** From mice immunized with purified muscarinic receptors, six hybridomas secreting monoclonal antibodies specific for muscarinic cholinergic receptors were derived from two cell fusions, each producing hybridomas in 300 out of 300 microtiter wells. Anti-muscarinic receptor monoclonal antibodies were identified by indi-

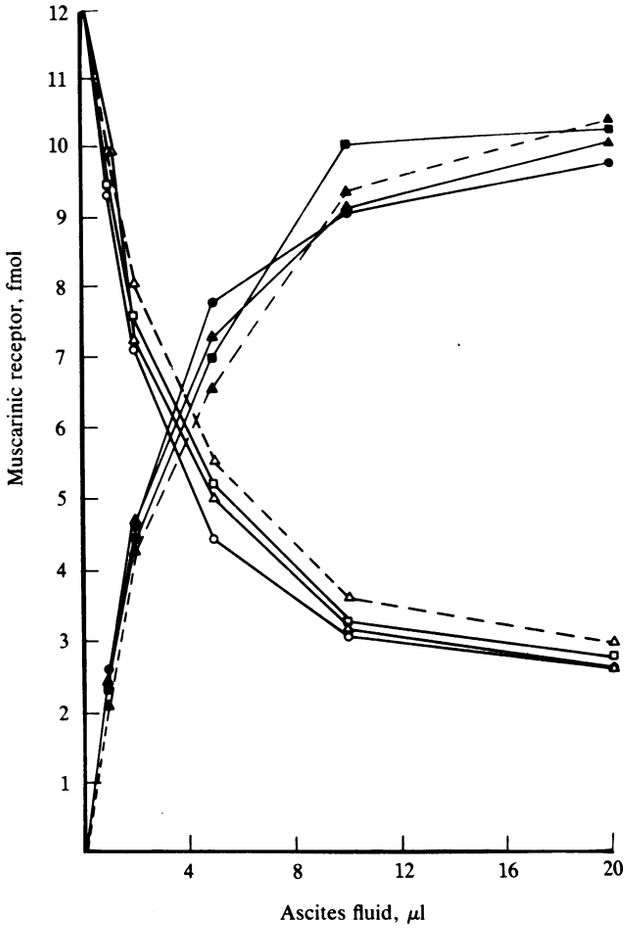


FIG. 3. Monoclonal antibody immunoprecipitation of muscarinic cholinergic receptors. Rat brain muscarinic receptors were affinity labeled with [$^3$H]PrBCM, solubilized with 1% $NaDodSO_4$, and partially purified by a combination of isoelectric focusing as described for Fig. 2, followed by gel permeation chromatography (Sephacryl S-300, 2.5 × 65 cm, 20°C equilibrated with 0.1% $NaDodSO_4$). Muscarinic receptor (12 fmol) was incubated with the indicated volume of ascites fluid from hybridoma M1.6 (●—●, ○—○), M1.42 (▲—▲, △—△), M1.43 (■—■, □—□), and M1.44 (▲---▲, △---△) in a total volume of 500 $\mu$l at 20°C for 4 hr, at which time anti-mouse IgG was added and the reaction was continued for an additional 12 hr. The reactions were terminated by centrifugation in a microcentrifuge (5 min). Open symbols represent the loss of receptor from supernates and filled symbols represent the appearance of receptor in the immune complex pellets. Experiments with control mouse IgG or control ascites fluid showed no loss of receptor from supernates or appearance of receptor in pellets. One microliter of ascites fluid contained the following amounts of IgG: 1.0 $\mu$g (M1.6); 1.2 $\mu$g (M1.42); 1.6 $\mu$g (M1.43); and 1.1 $\mu$g (M1.44); each was ≈50% monoclonal antibody.

Table 2. Immunoprecipitation of $\alpha_1$-adrenergic and cholinergic receptors by monoclonal antibodies for muscarinic cholinergic and $\alpha_1$-adrenergic receptors

| Antigen | Monoclonal antibody | Isotype* | Receptor: Muscarinic: Rat brain | Human brain | Dog heart | Monkey ciliary muscle | *Drosophila* head | $\alpha_1$-Adrenergic: Rat liver |
|---|---|---|---|---|---|---|---|---|
| Purified rat brain muscarinic receptor | M1.1 | IgG2a | + | + | + | + | + | − |
| | M1.6 | IgG2b | + | + | + | + | + | + |
| | M1.30 | IgG2a | + | + | + | + | + | − |
| | M1.42 | IgG1 | + | + | + | + | + | − |
| | M1.43 | IgG2a | + | + | + | + | + | − |
| | M1.44 | IgG1 | + | + | + | + | + | − |
| Rat liver membranes | $\alpha_1$-IVC$_2$ | IgG1 | + | ND | + | + | ND | + |
| | $\alpha_1$-IVE$_3$ | IgG2b/2a | + | ND | + | + | ND | + |
| | $\alpha_1$-IF$_9$ | IgG2a | − | ND | − | − | ND | + |
| | $\alpha_1$-IIIF$_3$ | IgG1 | − | ND | − | − | ND | + |
| | $\alpha_1$-ID$_8$ | IgG2a | − | ND | − | − | ND | + |

Data indicate the ability of monoclonal antibodies to immunoprecipitate affinity-labeled receptors as described for Fig. 3 relative to the data obtained with rat brain receptors (which were used as a source of antigen). For muscarinic receptors, + indicates 100% crossreactivity with a fixed amount (1.0 $\mu$g of IgG) of each antibody with 2.0 fmol of purified receptor. For $\alpha_1$-adrenergic receptors, + indicates the ability of monoclonal antibodies (1 $\mu$g of IgG) to immunoprecipitate [$^3$H]POB-labeled $\alpha_1$-adrenergic receptors (2.0 fmol). ND, not determined.
*Isotype determined by using spent culture media and the Ouchterlony double-diffusion test.

rect immunoprecipitation of the affinity-labeled receptor molecules (Fig. 3). This screening technique excluded the detection of antibodies specific for the ligand binding site. Each clone positive for muscarinic receptors (Table 2) originated in a separate well of the microtiter plates, and further cloning by limiting dilution was performed to ensure antibody homogeneity. Curves for indirect immunoprecipitation of affinity-labeled muscarinic cholinergic receptors (Fig. 3) demonstrate the specificity and apparent affinity of monoclonal antibodies (from ascites fluid) for the purified receptor. The properties of the monoclonal antibodies specific for muscarinic receptor are summarized in Table 2.

**Production and Characterization of Monoclonal Antibodies Specific for $\alpha_1$-Adrenergic Receptor.** From mice immunized with rat liver plasma membranes, five hybridomas secreting monoclonal antibodies specific for $\alpha_1$-adrenergic receptors were derived from two cell fusions, each producing hybridomas in 300 out of 300 wells. Anti-$\alpha_1$-adrenergic receptor monoclonal antibodies were also identified by indirect immunoprecipitation of partially purified affinity-labeled adrenergic receptors (Table 2). Each clone positive for $\alpha_1$-receptors (Table 2) originated in a separate well of the microtiter plate and further cloning by limiting dilution was performed to ensure antibody homogeneity.

**Monoclonal Antibody Crossreactivity.** Muscarinic receptors were affinity labeled with [$^3$H]PrBCM, solubilized with detergent, and partially purified by preparative isoelectric focusing. The pI 5.9 peak for each receptor was utilized in immunoprecipitation studies. The six monoclonal antibodies specific for muscarinic receptor immunoprecipitated muscarinic receptors from all sources tested, including human and rat brains and *Drosophila* heads as well as cardiac and ciliary muscle, with equal efficacy (Table 2).

One monoclonal antibody, M1.30, raised and screened against muscarinic cholinergic receptors was able to selectively immunoprecipitate partially purified affinity-labeled $\alpha_1$-adrenergic receptors, whereas the other five muscarinic receptor monoclonal antibodies were without effect (Table 2). In contrast, two out of the five monoclonal antibodies raised and screened against rat liver $\alpha_1$-adrenergic receptors, $\alpha_1$-IVC$_2$ and $\alpha_1$-IIIE$_3$, were able to immunoprecipitate muscarinic cholinergic receptors isolated from rat brain, dog heart, and monkey ciliary muscle with equal efficacy (Table 2).

## DISCUSSION

The 80,000-dalton NaDodSO$_4$/polyacrylamide gel electrophoretic molecular mass and common isoelectric point (pI = 5.9) of muscarinic cholinergic receptors isolated from human and rat brains and *Drosophila* heads, dog heart, and monkey ciliary muscle (Figs. 1 and 2 and Table 1) indicate structural similarities between these receptors. Further support for structural relatedness of these receptors is derived from monoclonal antibody crossreactivity data (Table 2), in which six out of six anti-muscarinic receptor antibodies demonstrated 100% crossreactivity with muscarinic receptors from all tissues tested, from human brain to *Drosophila* heads (Table 2). These data suggest that muscarinic cholinergic receptors are highly conserved over a substantial period of evolution.

That diverse expression of muscarinic receptor function is found in lower species such as molluscs, in which acetylcholine both attenuates heart rate and stimulates the central nervous system (3, 4), together with the structural similarities in the receptors demonstrated here and previously (1), supports the concept that only one major structural form of the receptor may exist (1). Response diversity found with muscarinic receptors in different tissues may therefore be the result of interactions between the receptor protein and different effector molecules in the plasma membrane.

Despite the fact that muscarinic cholinergic and $\alpha_1$-adrenergic receptors are associated with opposing sides of the autonomic nervous system and respond to unique neurotransmitters *in vivo*, muscarinic cholinergic and $\alpha_1$-adrenergic receptors have been shown to modulate a number of common effector proteins such as the slow inward calcium channel (19). Consequently, it would not be surprising to find similar or identical regions within each class of receptor that are involved in the interactions between these receptors and other membrane proteins. Tryptic digestion experiments have indicated superficial similarities between muscarinic cholinergic and $\alpha_1$-adrenergic receptors (1, 9, 10). However, monoclonal antibody crossreactivity between these two diverse pharmacological classes of receptors provides direct evidence for possible common receptor determinants. We propose that some degree of structural homology will be found among the diverse group of peptide hormone and neurotransmitter receptors that mediate responsiveness via the

calcium channel and adenylate cyclase systems.

While structural similarities appear to exist between $\alpha_1$-adrenergic and muscarinic cholinergic receptors, substantial differences should also be noted. The $\alpha_1$-adrenergic receptor exists as a dimer in the membrane with a molecular mass of 160,000 daltons, in contrast to the monomeric form (80,000 daltons) of the muscarinic acetylcholine receptor (1, 9, 10). The isoelectric points of these receptors differ, 3.7 for the $\alpha_1$-adrenergic receptor vs. 5.9 for the muscarinic receptor. Five out of six monoclonal antibodies specific for the muscarinic receptor did not crossreact with the $\alpha_1$-receptor and three out of five anti-$\alpha_1$-receptor antibodies did not crossreact with the cholinergic receptor (Table 2). However, these findings raise the possibility that there are conserved regions of the receptor proteins (sites of monoclonal antibody crossreactivity) among diverse pharmacological classes and that these domains may be responsible for the interaction of the receptors with common membrane effector molecules.

This work was supported by National Institutes of Health Grants AI19346 and HL31178 to J.C.V., by American Heart Association Grant 82-881 to C.M.F., and by a grant from the Council for Tobacco Research–U.S.A., Inc., to L.M.H.

1. Venter, J. C. (1983) *J. Biol. Chem.* **258,** 4842–4848.
2. Kistler, J., Stroud, R. M., Klymkowsky, M. W., Lalancette, R. & Fairclough, R. H. (1982) *Biophys. J.* **37,** 371–383.
3. Michelson, M. J. (1974) *Biochem. Pharmacol.* **23,** 2211–2224.
4. Fisher, H. (1972) *Naturwissenschaften* **59,** 425–435.
5. Haim, N., Nahum, S. & Dudai, Y. (1979) *J. Neurochem.* **32,** 543–552.
6. Jones, S. W. & Sumikawa, K. (1981) *J. Neurochem.* **36,** 459.
7. Dudai, Y. & Ben-Barak, J. (1977) *FEBS Lett.* **81,** 134–136.
8. Kunos, G., Kan, W. H., Greguski, R. & Venter, J. C. (1983) *J. Biol. Chem.* **258,** 326–332.
9. Venter, J. C. & Fraser, C. M. (1983) *Trends Pharmacol. Sci.* **4,** 256–258.
10. Fraser, C. M., Greguski, R., Eddy, B. & Venter, J. C. (1983) *J. Cell. Biochem.* **21,** 219–231.
11. Fraser, C. M. & Venter, J. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7034–7038.
12. Gitschier, J., Strichartz, G. R. & Hall, L. M. (1980) *Biochim. Biophys. Acta* **595,** 291–303.
13. Giacobino, J. P., Kaul, R. K., Kohler, H., Eddy, B. & Venter, J. C. (1983) in *Receptor Biochemistry and Methodology,* eds. Venter, J. C. & Harrison, L. C. (Liss, New York), Vol. 2, in press.
14. Fraser, C. M. & Lindstrom, J. (1983) in *Receptor Biochemistry and Methodology,* eds. Venter, J. C. & Harrison, L. C. (Liss, New York), Vol. 3, in press.
15. Lilly, L., Eddy, B., Schaber, J. S., Fraser, C. M. & Venter, J. C. (1983) in *Receptor Biochemistry and Methodology,* eds. Venter, J. C. & Harrison, L. C. (Liss, New York), Vol. 2, in press.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Haga, T. (1980) *FEBS Lett.* **113,** 68–72.
18. Birdsall, N. J. M., Burgen, A. S. V. & Hulme, E. C. (1979) *Br. J. Pharmacol.* **66,** 337–342.
19. Venter, J. C., Fraser, C. M., Schaber, J. S., Jung, C. Y., Bolger, G. & Triggle, D. J. (1983) *J. Biol. Chem.* **258,** 9344–9348.