Preparation and characterization of muscarinic-acetylcholine-receptor-enriched membranes from pig atria

Gary L. PETERSON and Michael I. SCHIMERLIK

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

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A procedure was developed for the large scale preparation of membranes from pig atria which are enriched 10–13-fold in the muscarinic acetylcholine receptor. The procedure involved differential centrifugation and sucrose-gradient centrifugation in solutions containing 150 mM-NaClO₄ and 5 mM-EDTA to minimize membrane aggregation. The final membrane preparation bound about 1.1 pmol of L-quinuclidinyl benzilate/mg of protein. Comparable results were obtained with either fresh or frozen tissue. About the same yield (120 pmol of L-quinuclidinyl benzilate sites/100 g of tissue) and specific activity of membranes were obtained from different regions of the atria. The final preparation was stable at –80°C in buffered sucrose solutions. The membranes appeared mostly as sheets or fragments and partly as closed vesicles in the electron microscope and were heterogeneous in isopycnic Percoll gradients. Marker enzyme studies showed that the receptor was enriched in parallel with the plasma membrane markers guanylate cyclase (particulate form) and (Na⁺ + K⁺)-activated ATPase. Some contamination by mitochondrial outer and endoplasmic reticulum membranes was evident from the distribution of monoamine oxidase and glucose-6-phosphatase activity, but the preparation was largely free of sarcoplasmic reticulum, mitochondrial inner, and lysosomal membranes.

The muscarinic acetylcholine receptor is found in relatively high concentrations in brain (Yamamura & Snyder, 1974a) smooth muscle (Yamamura & Snyder, 1974b) and myocardium (Fields *et al.*, 1978). The binding characteristics of agonists and antagonists in these and other tissues from several species appear to be similar.

In this laboratory we have chosen to use pig atria as a source of muscarinic receptor in studies aimed at the purification of this receptor and identification of its structure–function relationships. In approaching the purification of the pig atrial receptor, it was desirable to optimize the yield and relative purity of the receptor in the membrane state to serve not only as an improved starting point for the solubilization step, but also as a high activity reference source of normal or ‘native’ membrane-bound receptor. In this paper we describe the large scale preparation and characterization of a membrane fraction from pig atria enriched in specific L-[³H]quinuclidinyl benzilate binding capacity.

Experimental

Materials

L-[³H]Quinuclidinyl benzilate (40.2 Ci/mmol) was purchased from New England Nuclear, and was

found to be over 90% pure (Cremo *et al.*, 1981). The cyclic GMP assay kit (cyclic GMP binding protein/cyclic [³H]GMP radioisotope dilution test) was obtained from Boehringer Mannheim and all other reagents were the highest purity commercially available. GF/B filter discs (24 mm) were purchased from Whatman. The commonly used solutions were prepared and defined as follows: solution SA, 250 mM-sucrose dissolved in buffer A; solution SB, 250 mM-sucrose dissolved in buffer B; buffer A, 150 mM-NaClO₄/5 mM-disodium EDTA/0.02 mg of NaN₃/ml/0.1 mM-phenylmethanesulphonyl fluoride/25 mM-imidazole/HCl, pH 7.4; buffer B, 1 mM-disodium EDTA/0.02 mg of NaN₃/ml/0.1 mM-phenylmethanesulphonyl fluoride/25 mM-imidazole/HCl, pH 7.4.

Preparation of atrial muscarinic receptor-enriched membranes

Atrial tissue, including the left and right smooth-walled atrium proper and the pectinate-muscle-lined atrium and auricle, was removed from pigs immediately after slaughter and kept on ice. At the laboratory, extraneous heart tissue, fat and blood were removed and the atrial tissue was either used directly or stored at –80°C. Typically, 100 g of fresh or thawed frozen tissue was cut into small

pieces and added to 450 ml of solution SA. The tissue was homogenized under argon at 4°C for 1 min at full speed using a Polytron homogenizer equipped with the PT35K probe. The homogenate (H) was centrifuged in a Sorvall GSA rotor for 15 min at 2°C and 400 *g* (r_{av} , 9.0 cm). The supernatant (S1) was filtered through four layers of cheesecloth, and then centrifuged in a Beckman type 35 rotor for 1 h at 2°C and 70 000 *g* (r_{av} , 7.0 cm). The resulting supernatant was decanted completely, removing in addition as much as possible of the overlying clear-appearing pellet layer (more prominent in fresh tissue) without disturbing the underlying pellet layer. The majority of the remaining buff-coloured pellet (P2) was removed with the aid of a bent glass rod, leaving behind a small dark brown button. The crude membrane or P2 fraction was resuspended in solution SA to a final volume of 120 ml by homogenization with a Polytron equipped with the PT10ST probe, at 30% of maximum speed for 10–15 s. The crude membranes were then centrifuged through a step gradient consisting of 10 ml of 28% (w/v) sucrose, 9 ml of 13% (w/v) sucrose and 20 ml of crude membrane suspension in Beckman SW28 cellulose nitrate tubes. The sucrose gradient solutions were prepared in buffer A. The gradients were centrifuged in a Beckman SW 28 rotor for 1 h at 2°C and 96 000 *g* (r_{av} , 11.83 cm). The membranes banding at the 13–28% sucrose interface were collected by aspiration, diluted with an equal volume of distilled water, and centrifuged in a Beckman type 35 rotor for 1 h at 2°C and 70 000 *g* (r_{av} , 7.0 cm). The resulting supernatant was completely decanted and the final membranes (P3) were resuspended in solution SB to a final protein concentration of 3–6 mg of protein/ml, using the Polytron and PT10ST probe as described above for the P2 fraction.

In some experiments the crude membrane or P2 fraction was further fractionated on linear sucrose density gradients rather than the step gradients described above. In these cases, 9 ml of the crude membranes (130 mg) were layered over 30 ml of 10–40% (w/v) linear sucrose gradients. Sucrose solutions were prepared in buffer A and the gradients were centrifuged exactly as described for the step gradients above. After centrifugation fractions (0.85–2.5 ml) were collected at 0.85 ml/min. The density of the fractions was determined with a Zeiss Abbé refractometer.

The gradient membranes (P3 fraction) were further fractionated in isopycnic density gradients of Percoll. The Percoll gradients were formed during centrifugation in a Beckman type 40 rotor for 15 min at 2°C and 70 000 *g* (r_{av} , 5.9 cm). The gradients consisted of 1.25 ml or 1.5 ml of Percoll (density 1.130 g/ml) and 4.5 mg of gradient membrane protein in a final volume of 9 ml of the same

composition as solution SB. Additional experiments were performed with these gradients in which an effort was made to alter specifically the density of the plasma membranes by digitonin treatment as described by Ives *et al.* (1980), or the density of vesicles containing the Ca²⁺-pump by loading them with oxalate (Greaser *et al.*, 1969).

Stability tests

For tests of stability during storage, a gradient membrane fraction with a quinuclidinyl benzilate binding capacity of 1.35 pmol/mg was repelleted in a Beckman type 35 rotor as described above and resuspended at a protein concentration of 2.6 mg/ml in either solution SA, solution SB, 10 mM-imidazole/HCl (pH 7.4)/1 mM-disodium EDTA or 10 mM-sodium phosphate buffer (pH 7.4)/1 mM-disodium EDTA. The membrane suspensions were then stored in small aliquots at 0°C (ice), –20°C and –80°C, and sampled periodically over a 2-month period for quinuclidinyl benzilate binding activity.

Electron microscopy

The gradient membranes (P3 fraction) were examined by negative staining and thin-sectioning techniques. For negative staining, membranes adhered to Formvar-coated grids were stained with 1% phosphotungstic acid and examined at 60 kV ($\times 8000$) with a Philips 200 electron microscope. Samples were prepared for thin-section electron microscopy using methods similar to those of St. Louis & Sulakhe (1976) and Baker & Potter (1980). The sections were examined with the Philips 200 electron microscope at $\times 5000$ – $\times 19000$.

Assay methods

The muscarinic acetylcholine receptor was quantified in terms of L-[³H]quinuclidinyl benzilate binding sites as described by Yamamura & Snyder (1974*a,b*). Protein was measured by the method of Peterson (1977) with crystalline bovine serum albumin as the standard.

Guanylate cyclase activity was assayed under reaction conditions similar to those described by Kimura & Murad (1974) for the particulate enzyme of the rat heart. Cyclic GMP was directly assayed by the cyclic GMP-dependent protein binding radioisotope dilution method using the test kit purchased from Boehringer Mannheim.

Three ATPase enzymes were assayed: (Na⁺ + K⁺)-activated ATPase and NaN₃-insensitive and NaN₃-sensitive (Ca²⁺ + Mg²⁺)-activated ATPases. The (Na⁺ + K⁺)-activated ATPase activity was assayed by the method of Choi & Akera (1978), with (control) and without 0.1 mM-ouabain. The NaN₃-insensitive (Ca²⁺ + Mg²⁺)-activated ATPase activity was assayed in the presence of 0.5 mM-EGTA (control) or 50 μ M-CaCl₂ by the methods of

Hurwitz *et al.* (1973) and Hui *et al.* (1976). The NaN_3 -sensitive ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase activity was assayed as for the NaN_3 -insensitive form except that NaN_3 was omitted. With all the ATPases the enzyme reactions were run for 15 min at 37°C , and the amount of inorganic phosphate release was measured directly by the method of Peterson (1978).

Monomine oxidase activity was measured according to Schnaitman *et al.* (1967) using 2.5 mM-benzylamine hydrochloride as substrate. Enzyme activity was calculated from the A_{250} of the product benzaldehyde by using $\epsilon = 1.32 \times 10^4 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (Tabor *et al.*, 1954).

Glucose-6-phosphate activity was assayed by the methods of Kaulen *et al.* (1970) and Evans (1978). Sodium potassium tartrate (20 mM), disodium EDTA (5 mM) and NaF (1 mM) were added to inhibit other general phosphatases (Evans, 1978). Acid phosphatase activity was assayed by the method of Gianetto & de Duve (1955), except for the addition of Triton X-100 as suggested by Evans (1978) to activate latent activity. The reactions for glucose-6-phosphatase and acid phosphatase were run at 37°C and the activity was determined from the difference between the inorganic phosphate released (Peterson, 1978) at 90 min and at 30 min. The reaction remained linear when the time differential was varied up to 2 h.

Results and discussion

Table 1 shows a representative example of the preparation of muscarinic acetylcholine receptor-enriched membranes from the standard preparation of 100 g of frozen pig atria according to the methods described in the Experimental section. Usually about 40–50% of the $\text{L-}^3\text{H}$ quinuclidinyl benzilate binding sites present in the homogenate are recovered in the low-speed-supernatant fraction with little or no change in the specific activity. About 80% of the receptor sites in the low speed supernatant (S1) are recovered in the first high-speed pellet (P2) with about a 2–3-fold increase in the specific activity. About one-third of the receptor sites in the P2 fraction are recovered in the final gradient membrane fraction (P3) with about a 5-fold further enrichment in the receptor sites. The average ($\pm$ S.D.) yield for the P3 fraction from four different preparations of 100 g of tissue each was 118 (± 65) mg of total protein and 121 (± 38) pmol of total quinuclidinyl benzilate sites, with a specific activity of 1.13 (± 0.31) pmol of sites/mg of protein and a relative yield and purification of 11.1 (± 4.2)% and 11.4 (± 1.6)-fold, respectively. Very similar results are obtained with fresh atria (results not shown).

The specific binding capacity of these pig atrial membrane preparations compares very favourably

with that of other preparations in the literature. Wei & Sulakhe (1979) reported specific activities of about 0.4–0.5 pmol/mg in microsomal preparations of atria from rat, rabbit, guinea pig and dog. Specific activities in the range of 1–2 pmol/mg have been reported for membrane preparations from brain tissue (Carson *et al.*, 1977; Hurko, 1978; Uchida *et al.*, 1978; Ruess *et al.*, 1979; Gorissen *et al.*, 1981). Somewhat higher activity membrane preparations have been reported for rat brain tissue (Alberts & Bartfai, 1976; Gorissen *et al.*, 1981).

As many as 12 discrete membrane bands can be observed when freshly prepared gradient membranes are recentrifuged through Percoll gradients; however, no particular band was found to be markedly enriched in receptor sites. Perturbation of the density profile of the P3 membranes was achieved by use of digitonin (Ives *et al.*, 1980) and calcium oxalate loading (Greaser *et al.*, 1969) experiments, but with no accompanying separation of a fraction further enriched in muscarinic receptor sites.

Examination of the regional distribution of the receptor in the pig atrial tissue showed that the receptor density of the left atrium (10.8 pmol of quinuclidinyl benzilate bound/g of tissue) was about the same as that of the right atrium (9.9 pmol/g of tissue), but the smooth-walled 'atrium' proper region has a lower receptor density (7.2 pmol/g of tissue) than the 'auricle' region (13.6 pmol/g of tissue). Receptor density in the ventricles was found to be much lower, which is in agreement with the reduced parasympathetic innervation of this heart tissue and the data of others (Fields *et al.*, 1978; Wei & Sulakhe, 1979). The recovery of receptor in the gradient membrane fraction from the 'atria' was approx. 2-fold higher than from the 'auricles' (16.7 vs. 8.9%, respectively), which because of the lower 'atrial' receptor density resulted in approximately equivalent yields of receptor per g of tissue. The specific activities of the P3 fractions from the 'atrial' and 'auricle' region were also similar. Thus, there was no practical advantage in isolation of certain atrial regions for preparation of the muscarinic receptor-enriched membranes, and all further studies were carried out with both left and right whole atrial tissue.

Stability tests showed that at 0°C (ice) the muscarinic acetylcholine receptor was most stable in solutions not containing sucrose. Storage at 0°C resulted in an initial rapid loss of about 20% of the receptor sites within 2 weeks for all buffers tested. Over the subsequent 6-week experimental period there was an additional loss of about 5% of the original quinuclidinyl benzilate binding sites in 10 mM-imidazole, 10 mM-phosphate or solution SA, and about 30% in solution SB. At -20°C there was a near-linear time-dependent loss in receptor sites,

Table 1. *Preparation of pig atrial muscarinic acetylcholine receptor-enriched membranes and distribution of marker enzymes*

The fractionation procedure was performed as described in the Experimental section using 100g of frozen atrial tissue. All data shown are from a single membrane preparation. Total protein is expressed in mg, and total and specific activities for the receptor are expressed in pmol of quinuclidinyl benzilate bound and pmol bound/mg of protein, respectively. The units of guanylate cyclase total and specific activities are nmol of cyclic GMP/h and nmol of cyclic GMP/h per mg of protein, respectively. For all other enzymes the corresponding units are μ mol of product formed/h and μ mol of product formed/h per mg of protein. Relative yield and purification are calculated with respect to total and specific activities of the homogenate (H), and are in units of % and -fold, respectively.

Receptor or marker enzyme	Total protein	Total activity	Specific activity	Relative yield	Relative purification
Muscarinic acetylcholine receptor					
H	11 990	1020	0.085	100.0	1.00
S1	5300	528	0.100	51.8	1.17
P2	1800	363	0.202	35.7	2.38
P3	110	106	0.957	10.4	11.3
Guanylate cyclase					
H	—	7450	0.622	100.0	1.00
S1	—	4890	0.923	65.6	1.48
P2	—	1390	0.772	18.7	1.24
P3	—	301	2.74	4.0	4.41
(Na⁺ + K⁺)-activated ATPase					
H	—	1664	0.139	100.0	1.00
S1	—	777	0.147	46.7	1.06
P2	—	1230	0.684	73.9	4.94
P3	—	250	2.30	14.7	16.0
Monoamine oxidase					
H	—	226	0.019	100.0	1.00
S1	—	203	0.038	89.7	2.03
P2	—	65	0.036	28.6	1.91
P3	—	46	0.422	20.5	22.3
(Ca²⁺ + Mg²⁺)-activated ATPase, NaN₃-sensitive					
H	—	12 890	1.075	100.0	1.00
S1	—	4260	0.803	33.0	0.75
P2	—	1810	1.006	14.0	0.94
P3	—	318	2.89	2.4	2.60
(Ca²⁺ + Mg²⁺)-activated ATPase, NaN₃-insensitive					
H	—	12 310	1.027	100.0	1.00
S1	—	1980	0.374	16.1	0.36
P2	—	1033	0.575	8.4	0.56
P3	—	128	1.164	1.0	1.10
Glucose-6-phosphatase					
H	—	1213	0.101	100.0	1.00
S1	—	493	0.093	40.6	0.92
P2	—	359	0.200	29.6	1.97
P3	—	61	0.550	5.0	5.44
Acid phosphatase					
H	—	2215	0.185	100.0	1.00
S1	—	942	0.178	42.5	0.96
P2	—	529	0.294	23.9	1.59
P3	—	60	0.547	2.7	2.96

which after 2 months resulted in receptor losses of 70% in solution SA, 80% in the imidazole buffer solution, 90% in the phosphate buffer solution and 95% in solution SB. At -80°C 100% of the receptor sites remain after 1 month storage in either solution SA or SB, and after 2 months 90% of the sites remain when stored in solution SA and 95% when

stored in solution SB. Without sucrose present at -80°C , receptor loss continues slowly for about 1 month stabilizing at about 80% of the original sites for the imidazole buffer solution and 85% for the phosphate buffer solution. Thus the receptor from pig atria is best stored at -80°C in solution SB. The significance of the more labile fraction

of receptor sites is unknown. Possibly they represent a different conformational state of the receptor, or are derived from different locations in the cell membrane (e.g., pre-synaptic vs. post-synaptic membranes; Sharma & Banerjee, 1978), which impart different stability characteristics. Sodium dodecyl sulphate/polyacrylamide-gel analysis (Laemmli, 1970) of the membranes stored at 0°C vs. those stored at -80°C for 2 months revealed some minor changes in the protein profile, but most protein bands were identical in the two cases. In the samples stored at -20°C for 2 months (80–95% loss of binding sites) there were no obvious differences in the protein patterns in comparison with the samples stored at -80°C.

Negative-stained and thin-sectioned transmission electron photomicrographs of the gradient membrane fraction revealed principally membrane sheets and vesicular structures, similar to the sarcolemmal preparations shown previously by Kidwai *et al.* (1971), St. Louis & Sulakhe (1976), Bers (1979) and Baker & Potter (1980). No intact mitochondria could be found, although structures with projecting knobs could be seen occasionally in the negative-stained preparations. No evidence of contractile elements, nuclei or other recognizable cellular organelle was observed.

The distribution of the marker enzymes in the fractions obtained in the standard preparation of the receptor-enriched membranes is shown in Table 1, and their distribution in the linear sucrose gradients of the crude membrane (P2) fraction from the same experiment is shown in Fig. 1. The density profile of the linear sucrose gradient is shown in Fig. 1(a), and the fractions that would correspond to the 13–28% sucrose interface in the step gradient of the standard preparation procedure are indicated by the horizontal bar. The fractions enclosed by the horizontal bar (fractions 5–11) contain 12% of the total protein (Fig. 1a) and 41% of the total receptor sites, with a near symmetrical peak in muscarinic receptor specific activity distributed about fraction 8 (Fig. 1b). The gradient also contains a pellet which consists of 50% of the total protein and 40% of the total receptor on the gradient. Less than 2% of the total quinuclidinyl benzilate binding sites are observed in the upper portion of the gradient, which contains the light sedimenting particles and soluble proteins (estimated at 20%) remaining in the crude membrane fraction. Overall recoveries of protein and receptor sites in this gradient experiment were 90% and 91%, respectively.

The distribution of guanylate cyclase activity in the linear sucrose gradient experiment (Fig. 1c) suggested that about 20–25% of the total activity in the P2 fraction was due to a soluble form, and hence the yield of particulate guanylate cyclase in fractions P2 and S1 was about 15–20% of the total

homogenate activity. Therefore about half or more of the homogenate guanylate cyclase is of the soluble form, which is in agreement with the results reported by Kimura & Murad (1974) and by Sulakhe *et al.* (1976) for heart guanylate cyclase from several mammalian species. Thus, of the total particulate guanylate cyclase at least 30% and 8% are recovered in the P2 and P3 fractions, respectively, compared with 36% and 10% for the muscarinic receptor. In the linear sucrose gradient experiment, fractions 5–11 accounted for 41% of the estimated particulate guanylate cyclase activity compared with 41% for the muscarinic receptor distribution. Therefore, these two proteins appear generally to co-purify in the membrane state. Muscarinic receptor agonists elevate the tissue level of cyclic GMP in heart (George *et al.*, 1970); however, the precise role of this nucleotide in the agonist/muscarinic receptor-mediated events remains unclear (Nawrath, 1977; Linden & Brooker, 1979). The ($\text{Na}^+ + \text{K}^+$)-activated ATPase, another plasma membrane marker (see Fleischer & Parker, 1974; Evans, 1978) similarly appears to co-enrich with the muscarinic receptor (Table 1; Figs. 1b and 1d), in agreement with the studies of Ruess *et al.* (1979) using preparations from the bovine caudate nucleus.

Contamination of the preparation is principally due to mitochondrial outer membrane and endoplasmic reticulum. Some separation of these membranes from the muscarinic receptor-containing plasma membrane is achieved in the final gradient step, as shown by the lower relative abundance of monoamine oxidase (Fig. 1e) and glucose-6-phosphatase (Fig. 1g) in fractions 5–11 of the linear sucrose gradient as compared with the distribution of muscarinic receptor (Fig. 1b). For glucose-6-phosphatase, similar results were obtained with the sucrose step gradient (Table 1). For monoamine oxidase the results of Table 1 are not in apparent agreement with those of Fig. 1(e). However, overall recovery of monoamine oxidase activity on the linear gradient was 180%, indicating either that the P2 activity was underestimated or that the enzyme was in some way activated after sucrose-gradient centrifugation, which largely accounts for the discrepancy.

Sarcoplasmic reticulum and mitochondrial inner membranes are expected to be abundant in cardiac tissue. These membranes were separated and largely eliminated during preparation of the muscarinic receptor-enriched membranes. Only 1% of the NaN_3 -insensitive ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase (Ca^{2+} pump activity of the sarcoplasmic reticulum) was present in the P3 fraction with no purification over the homogenate (Table 1). The relative yield and purification of the mitochondrial inner membrane marker, NaN_3 -sensitive ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated

Fig. 1. Distribution of protein, muscarinic acetylcholine receptor and marker enzymes of the P2 fraction on a linear sucrose density gradient

The sucrose gradient consisted of 30 ml of 10–40% sucrose prepared in buffer B, with 9 ml (130 mg of protein) of crude membranes (fraction P2), and was centrifuged for 1 h in a Beckman SW 28 rotor as described in the Experimental section. This was the same fraction P2 as obtained in the experiment of Table 1. The fractions were collected at 3 min intervals at a flow rate of approx. 0.85 ml/min and gave a measured volume of 2.65 ml/fraction. The pellet which formed at the bottom of the gradient was resuspended in solution SB to a final volume of 10 ml. The density of each fraction was determined by refractometry and is shown by (○) in (a). The horizontal bar delineates the density range from the 13% sucrose solution to the 28% sucrose solution, which would be comparable with the 13–28% sucrose interface in the standard step gradient preparation. For reference the horizontal bar is repeated in each panel. ●, total protein, receptor or marker enzyme per fraction; ○ in (b)–(h), the specific activity of the receptor (b) or marker enzymes (c)–(h). Abbreviation used: QNB, quinuclidinyl benzilate.

ATPase, in fraction P3 were 4-fold lower than those values for the muscarinic receptor (Table 1), most of the mitochondrial inner membranes being heavier in sedimentation behaviour than those containing the

receptor (Table 1 and Fig. 1f). This agrees with the infrequent appearance of 'lollipop' structures and the absence of whole mitochondria as observed in the electron photomicrographs. The distribution of the

lysosomal marker, acid phosphatase, was similar to that of the mitochondrial inner membranes in the experiments of Table 1 and Fig. 1, indicating that lysosomal membranes were also separated and largely eliminated. These results are virtually identical to those reported by Bers (1979) for the neonatal whole rat heart in which the sarcolemma was enriched 10-fold by use of KCl pyrophosphate and sucrose gradients.

In conclusion, a procedure has been developed for the large scale preparation of muscarinic acetylcholine receptor-enriched membranes from pig atria. Either fresh or frozen tissue may be used and 100–200 g of tissue can be processed in 1 day. The preparation represents a 10–13-fold purification over the homogenate with a specific activity of 0.8–1.4 pmol of quinuclidinyl benzilate binding sites/mg of protein, which is as high or higher than most preparations from other tissue sources. The receptor is distributed in slightly greater density in the auricle sacs than in the atria proper, but left and right structures do not differ significantly from one another and the membrane preparations from each are essentially equivalent in yield and specific activity. The membrane preparations are stable for at least 2 months when stored at -80°C in the presence of 250 mM-sucrose/25 mM-imidazole/HCl (pH 7.4)/1 mM-EDTA. The preparation consists chiefly of membrane sheets or fragments as well as some closed vesicles, and is heterogeneous in density on isopycnic Percoll gradients. The receptor is enriched in parallel with the plasma membrane markers guanylate cyclase (particulate form) and ($\text{Na}^{+} + \text{K}^{+}$)-activated ATPase. Some contamination from mitochondrial outer and endoplasmic reticulum membranes is present, but the preparation is largely free of sarcoplasmic reticulum, mitochondrial inner and lysosomal membranes. These studies, therefore, provide the procedures for isolation of well-characterized and stable membranes from heart tissues which are enriched in the muscarinic acetylcholine receptor and serve as an excellent starting point for further purification efforts on the receptor itself.

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