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Effect of the Lipid Environment on the Differential Affinity of Purified Cerebral and Atrial Muscarinic Acetylcholine Receptors for Pirenzepine

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SUMMARY

Muscarinic acetylcholine receptors (mAChRs) of porcine cerebral membrane (predominantly M1 subtype) and porcine atrial membrane (M2 subtype) showed the same affinity for the muscarinic antagonist [^{3}H]quinuclidinylbenzylate ([^{3}H]QNB). In contrast, the affinity for pirenzepine (another muscarinic antagonist) of 86% of binding sites in the cerebral membrane (H sites) was 34-fold higher than that in the atrial membrane. After purification of mAChRs by affinity chromatography, this difference was less than 3-fold. This phenomenon was fully reversed by insertion of purified mAChRs into either cerebral or atrial membranes whose native muscarinic binding sites had been alkylated with propylbenzilylcholine mustard, indicating that the purified receptors recovered their original affinities for pirenzepine upon interaction with membrane components. To examine the effect of the interaction between receptors and lipid components on the affinities for [^{3}H]QNB and pirenzepine, binding experiments were carried out with mAChRs inserted into various lipid preparations. When purified cerebral and atrial mAChRs were inserted into cholesteryl hemisuccinate, their affinities for [^{3}H]QNB and pirenzepine became close to the membrane values and were 7- and 50- to 60-fold higher than those of receptors inserted into phosphatidylcholine, respectively. When insertion was carried out into either cholesteryl hemisuccinate, phosphatidylcholine, or cholesteryl hemisuccinate/phosphatidylcholine mixtures, (80:20 and 50:50, w/w), the affinity of cerebral H sites for pirenzepine was only 3- to 5-fold higher than that of atrial receptors, but it became 20- and 60-fold higher when the receptors were inserted in a cholesteryl hemisuccinate/phosphatidylcholine mixture (20:80, w/w) and in a cholesteryl hemisuccinate/phosphatidylcholine/phosphatidylinositol mixture (4:48:48, w/w), respectively. These results suggest that the affinities of mAChRs for antagonists, in particular the differential affinities of cerebral and atrial mAChRs for pirenzepine, are modulated by the lipid environment.

mAChRs are classified into pharmacological subtypes on the basis of their affinities for discriminatory antagonists such as pirenzepine. High affinity sites for pirenzepine (M1 subtype) are abundant in the mammalian cerebral cortex and sympathetic ganglia, whereas low affinity sites (M2 subtype) are abundant in hindbrain, atrium, smooth muscle, and exocrine glands (1–3). Molecular cloning, sequencing, and expression of either cDNAs or genes encoding mAChRs have indicated that the pharmacologically defined subtypes correspond to different polypeptides (4–10) and that there are at least five different mAChR subtypes (7–9, 11).

The ability of pirenzepine to discriminate between the M1 and M2 subtypes is greatly reduced after solubilization (12–16) and purification (13, 17–19) of mAChRs, raising the possibility that membrane constraints may be involved in the affinity of mAChRs for this ligand.

To test this possibility, mAChRs that were purified from porcine cerebrum and atrium were inserted into either membrane preparations or lipid mixtures and then their binding to pirenzepine and to a nondiscriminatory antagonist ([^{3}H]QNB) was examined.

Experimental Procedures

Materials. [^{3}H]QNB (39 Ci/mmol) was purchased from Amersham Japan (Tokyo). Cholesteryl hemisuccinate (Tris salt), egg L-α-phosphatidylcholine (type III-E), and soybean L-α-phosphatidylinositol (ammonium salt) were obtained from Sigma. PrBCM was purchased from Funakoshi Yakuhin (Tokyo, Japan). Pirenzepine was kindly donated by Boehringer Ingelheim, Japan.

Purification of mAChRs. Porcine cerebral membranes (typically

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ABBREVIATIONS: mAChR, muscarinic acetylcholine receptor; [^{3}H]QNB, [^{3}H]quinuclidinylbenzylate; PrBCM, propylbenzilylcholine mustard; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; H sites, high affinity binding sites for pirenzepine; L sites, low affinity binding sites for pirenzepine; G protein, GTP-binding protein.

20 mg of protein/ml, 20 pmol of [^{3}H]QNB binding sites/ml) were prepared from the cortex and caudate nucleus, as described by Haga and Haga (20). Porcine atrial membranes (typically 5 mg of protein/ml, 7.5 pmol of [^{3}H]QNB binding sites/ml) were prepared as described by Peterson and Schimerlik (21). The membranes were stored at −80°. mAChRs were solubilized and purified by a single affinity chromatography, from either cerebral or atrial membrane preparations, as described in Refs. 19 and 20.

Alkylation of mAChRs in membrane preparations. Frozen cerebral (75 μl) or atrial membranes (1 ml) were thawed, diluted to 1 mg of protein/ml with 20 mM potassium phosphate buffer (pH 7.0), and then incubated at 30° for 20 min in the presence of 150 nM PrBCM (an irreversible muscarinic antagonist). This procedure resulted in a loss of 93 to 95% of the [^{3}H]QNB binding activity. After incubation, 1 μM sodium thiosulfate was added to quench the unreacted aziridinium ion of PrBCM (22) and then the membranes were washed twice with 20 mM potassium phosphate buffer (pH 7.0) by centrifugation at 60,000 × *g* for 15 min at 4°. The final pellet was resuspended in ice-cold HEN medium which consisted of 20 mM potassium HEPES buffer (pH 7.5) containing 1 mM EDTA and 160 mM NaCl, and was then kept on ice.

Insertion of purified mAChRs into either PrBCM-treated membranes or lipid mixtures. The insertion was carried out essentially as described by Florio and Sternweis (23). PrBCM-treated membranes (0.5 to 1.5 mg of protein) were mixed with 50 mM oxotremorine, 0.18% sodium deoxycholate, and 0.04% sodium cholate in HEN, in a final volume of 1 ml, and were then sonicated under nitrogen with a bath-type sonicator (Branson B-220) at 2° for 15 min. Then, 10 to 15 μl of purified mAChR preparation (3 to 6 pmol of [^{3}H]QNB binding activity) were added, and the resultant suspension was mixed well with a vortex mixer and then subjected to gel filtration on Sephadex G-50 (fine), which had been preequilibrated with HEN. The void-volume fraction was collected and used as the inserted mAChRs. Alternatively, cholesteryl hemisuccinate, egg L-α-phosphatidylcholine, and soybean L-α-phosphatidylinositol were mixed in various proportions. After the solvent was evaporated under a stream of nitrogen, the lipid mixtures were resuspended in ice-cold HEN that contained 1% sodium deoxycholate, the final total lipid concentration being 4 mg/ml, and were then sonicated as described above until the suspension became transparent. Then, a mixture of 10 to 20 μl of purified mAChR preparation and 90 μl of HEN containing 100 mM oxotremorine were mixed with 100 μl of the lipid mixture. The resulting suspension was vortexed and then subjected to gel filtration as described above.

Binding assays and data analysis. The [^{3}H]QNB binding activity of purified receptors in solution was assayed as described previously (24). Saturation binding of [^{3}H]QNB and competitive binding between the radioligand and pirenzepine in either native membrane preparations or inserted receptors were carried out essentially as described by Florio and Sternweis (23). Receptor samples (50 μl) were mixed with 1 ml of a buffer solution that contained 25 mM potassium phosphate buffer (pH 7.0), 4 mM potassium HEPES (pH 7.5), 10 mM $MgCl_2$, 230 mM NaCl, 1 mM EDTA, 0.6 mg/ml bovine serum albumin, and various concentrations of muscarinic ligands. [^{3}H]QNB was used at a concentration of 0.6 nM for competition experiments and at a concentration range of 30 to 700 pM for the saturation binding experiments, except for the cases of receptors inserted in cholesteryl hemisuccinate/phosphatidylcholine (20:80, w/w) and phosphatidylcholine, in which concentration ranges of 30 to 1000 pM and 0.3 to 4 nM were used, respectively. After incubation at 30° for 1 hr, the samples were placed on ice and then filtered through Whatman GF/C filters, immediately followed by three washes with 1 ml each time of ice-cold 25 mM potassium phosphate buffer (pH 7.0) that contained 230 mM NaCl. The filters were dried and then counted in a Triton X-100/toluene (30:70, v/v) mixture that contained 0.4% (w/v) 2,5-diphenyloxazole and 0.01% (w/v) 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, with an efficiency of 38%. Specific binding was defined as the difference between the binding in the presence and that in the absence of 1 μM atropine sulfate. The data were subjected to a computer-assisted nonlinear regression analysis called SALS (Statistical Analysis with Least-Squares Fitting) (25) and analyzed as described previously (26). Saturation binding of [^{3}H]QNB fitted the following one-site model equation,

$$Y = \frac{B_{max} \times X}{K_d^* + X}$$

where Y is the specific binding at a concentration of [^{3}H]QNB equal to X, B_{max} is the total number of binding sites, and K_d^* is the dissociation constant of [^{3}H]QNB. The displacement curves from the competitive binding between [^{3}H]QNB and pirenzepine fitted either a one-site model (eq. a) or a two-site model (eq. b),

$$Y = \frac{100 \times IC_{50}}{X + IC_{50}} \quad (a)$$

$$Y = \frac{A \times IC_{50_H}}{X + IC_{50_H}} + \frac{(100 - A) \times IC_{50_L}}{X + IC_{50_L}} \quad (b)$$

where Y is the specific binding of [^{3}H]QNB at a concentration of pirenzepine equal to X, IC_{50} is the concentration of pirenzepine at which the specific binding of [^{3}H]QNB is the 50% of that in the absence of pirenzepine, IC_{50_H} and IC_{50_L} are the IC_{50} values of the H and L sites, respectively, and A is the percentage of the H sites. The dissociation constants for pirenzepine were calculated with the following equation (27),

$$K_d = \frac{IC_{50}}{1 + \frac{[[^3H]QNB]}{K_d^*}}$$

where K_d is the dissociation constant for pirenzepine. The recovery of [^{3}H]QNB binding sites was highest after insertion into either PrBCM-treated membranes (65 to 75%) or cholesteryl hemisuccinate/phosphatidylcholine mixtures (20:80, w/w) (53 to 57%). In the other cases, the recovery ranged from 16 to 30% (Fig. 1).

Results

The affinities of intact membrane preparations for [^{3}H]QNB and pirenzepine were determined on the basis of the saturation binding of [^{3}H]QNB and the competitive binding of the radioligand and pirenzepine, respectively. Intact cerebral and atrial membrane preparations showed essentially the same affinity for [^{3}H]QNB, the dissociation constants (K_d values) being 44 and 43 pM, respectively (Table 1). In contrast, the affinity for pirenzepine of cerebral membranes was higher than that of atrial membranes. The displacement by pirenzepine of [^{3}H]QNB binding of cerebral membranes fitted a theoretical curve, assuming the presence of two populations of binding sites with different affinities. H sites accounted for 86% of the total number of binding sites. The K_d of H sites was 23 nM and that of L sites was 650 nM. In the case of atrial membranes, the displacement curve fitted a single-site model equation and the K_d (790 nM) was 34-fold higher than that of cerebral H sites (Fig. 2 and Table 1). Cerebral H sites and atrial binding sites correspond to the M1 and M2 subtypes of mAChRs, respectively (1–3). After solubilization and purification by affinity chromatography, the affinities for pirenzepine of cerebral and atrial mAChRs in digitonin solution were similar to each other, the K_d values being 300 and 690 pM, respectively. In this case, the affinities for [^{3}H]QNB of cerebral and atrial preparations were 330 and 380 nM, respectively (18, 19).

To examine the reversibility of this phenomenon, purified mAChR preparations were inserted into membranes whose native mAChRs had been alkylated with PrBCM. The inserted mAChRs recovered their original membrane affinities for both

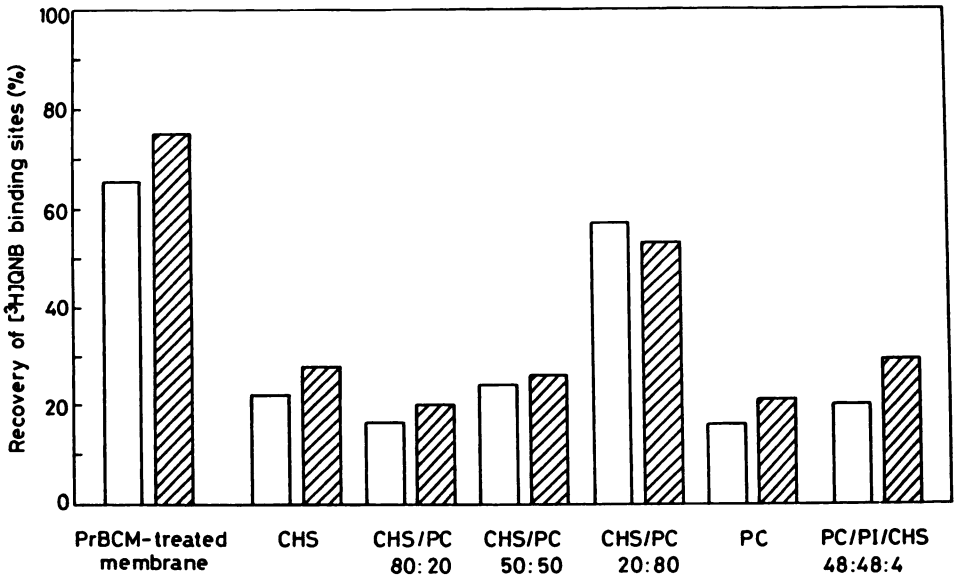

Fig. 1. Recovery of [^{3}H]QNB binding sites after insertion of mAChRs purified from cerebrum (□) or atrium (▨) into either PrBCM-treated membranes or lipid mixtures. In each case, the total number of [^{3}H]QNB binding sites of the inserted receptors was determined by means of saturation binding of the radioligand, followed by nonlinear regression analysis, as described under Experimental Procedures. Values are expressed as percentages of the [^{3}H]QNB binding activity of purified receptors in solution, which was determined by gel filtration, as described previously (23). *CHS*, cholesteryl hemisuccinate; *PC*, egg L-α-phosphatidylcholine; *PI*, soybean L-α-phosphatidylinositol.

TABLE 1

Affinities for [^{3}H]QNB and pirenzepine of native mAChRs in intact membranes and of mAChRs inserted into either PrBCM-treated membranes or lipid mixtures

The values are mean ± standard error obtained from three (*) or four (*) individual experiments, each of which was carried out in duplicate and analyzed as described under Experimental Procedures; [c] values are taken from Refs. 17 and 19. *CHS*, *PC*, and *PI*, cholesteryl hemisuccinate, egg L-α-phosphatidylcholine, and soybean L-α-phosphatidylinositol, respectively. The lipid to lipid ratios are given in w/w.

Environment	[^{3}H]QNB, K_d		Pirenzepine				
			Cerebrum			Atrium, K_d	$K_{d_{atrium}} / K_{d_{cerebrum\ H\ sites}}$
	Cerebrum	Atrium	Proportion of H sites	K_d H sites	K_d L sites		
	pM		%	nM		nM	
Native mAChR, Intact membrane[b]	44 ± 5	43 ± 3	86 ± 5	23 ± 6	650 ± 45	790 ± 30	34 ± 7
Inserted mAChR							
PrBCM-treated cerebral membrane[b]	44 ± 6	59 ± 7	79 ± 4	26 ± 4	730 ± 240	980 ± 63	38 ± 6
PrBCM-treated atrial membrane[b]	78 ± 11	72 ± 13	81 ± 3	30 ± 4	1,200 ± 125	1,275 ± 143	43 ± 7
CHS[a]	70 ± 13	81 ± 12	100 ± 1	56 ± 2		220 ± 34	4 ± 1
CHS:PC (80:20)[a]	74 ± 17	82 ± 25	100 ± 1	140 ± 29		440 ± 73	3 ± 0.2
CHS:PC (50:50)[a]	75 ± 21	81 ± 28	100 ± 1	270 ± 49		830 ± 80	3 ± 1
CHS:PC (20:80)[a]	130 ± 26	140 ± 19	75 ± 3	100 ± 16	2,200 ± 72	1,900 ± 138	19 ± 5
PC[a]	530 ± 75	590 ± 65	72 ± 7	2,700 ± 480	24,300 ± 1,430	13,500 ± 1,290	5 ± 2
CHS:PC:PI (4:48:48)[b]	170 ± 33	240 ± 53	73 ± 3	85 ± 15	4,300 ± 220	5,200 ± 270	61 ± 14
Isolated mAChR							
Digitonin solution[c]	330	380	100	300		690	2

[^{3}H]QNB and pirenzepine, regardless of whether cerebral or atrial membranes were used (Fig. 2 and Table 1); approximately 80% of the cerebral mAChRs showed 30- to 40-fold higher affinity than the remaining 20% in both cerebral and atrial membranes, and the atrial receptors only showed low affinity, regardless of which membranes were used. These findings indicate that the differential affinities of mAChRs for pirenzepine are modulated by the interaction between the receptors and membrane components.

To examine the effect of interaction between receptors and lipid components on the affinities for [^{3}H]QNB and pirenzepine, binding experiments were carried out with mAChRs that were inserted into various lipid mixtures.

After purified mAChRs had been inserted into cholesteryl hemisuccinate, the affinities of both cerebral and atrial receptors for [^{3}H]QNB and of cerebral receptors for pirenzepine were close to the membrane values, and the affinity of atrial receptors for pirenzepine was only 4-fold lower than that of cerebral receptors (Fig. 3 and Table 1). These findings suggest that the direct interaction between mAChRs and cholesteryl hemisuccinate results in high affinities of the receptors for these antagonists.

The affinities for [^{3}H]QNB and pirenzepine of receptors inserted into phosphatidylcholine were 7- and 50- to 60-fold lower than those of receptors inserted into cholesteryl hemisuccinate, respectively (Fig. 3 and Table 1).

When insertion was carried out into either cholesteryl hemisuccinate, phosphatidylcholine, or cholesteryl hemisuccinate/phosphatidylcholine mixtures (80:20 and 50:50 w/w), the affinity of cerebral receptors for pirenzepine was only 3- to 5-fold higher than that of atrial receptors. However, with the cholesteryl hemisuccinate/phosphatidylcholine ratio of 20:80 (w/w), the K_d of atrial mAChRs (1895 nM) became 19-fold higher than that of cerebral H sites (100 nM), which accounted

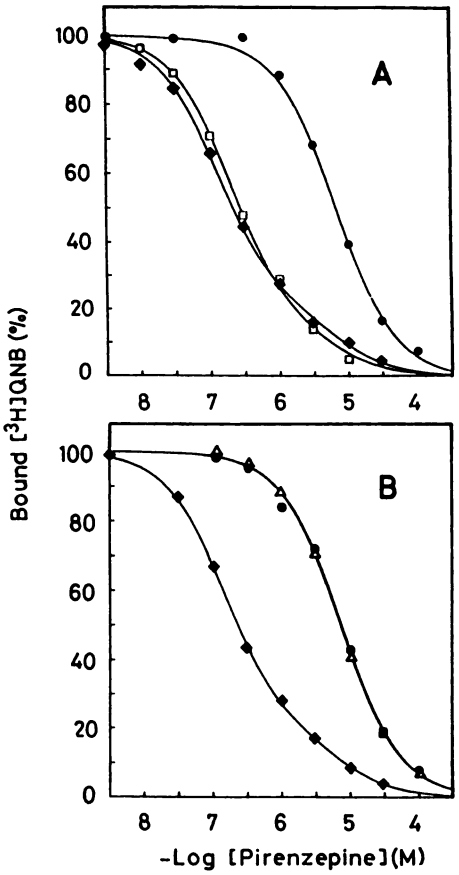

Fig. 2. Displacement by pirenzepine of [^{3}H]QNB binding of mAChRs in intact cerebral (□) or atrial (△) membranes and mAChRs purified from cerebrum (♦) or atrium (●) and then inserted into PrBCM-treated cerebral (A) or atrial (B) membranes. Competition experiments were carried out as described under Experimental Procedures. Specific [^{3}H]QNB binding in the absence of pirenzepine was taken as 100%, and the actual counts ranged from 1500 to 2000 cpm. The displacement curves were analyzed assuming the presence of either one (△, ●) or two (□, ♦) populations of binding sites, as described previously (26). The K_d values and proportions of H sites and L sites are presented in Table 1.

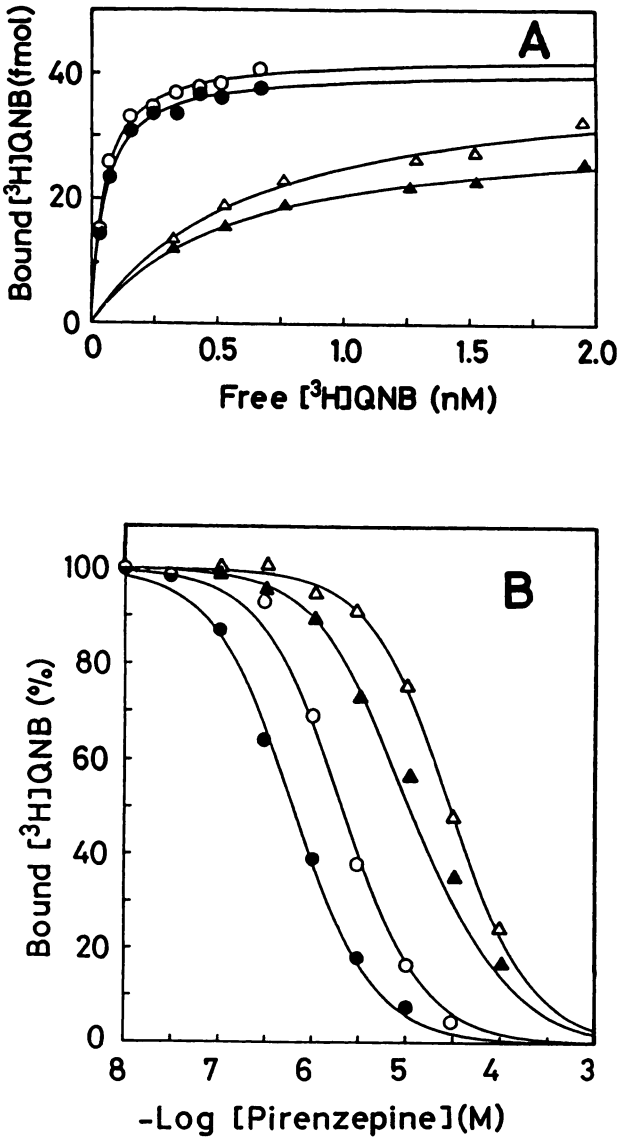

Fig. 3. [^{3}H]QNB binding (A) and displacement by pirenzepine of [^{3}H]QNB binding (B) of mAChRs purified from cerebrum (*closed symbols*) or atrium (*open symbols*), after insertion into either cholesteryl hemisuccinate (*circles*) or phosphatidylcholine (*triangles*). Saturation binding of [^{3}H]QNB was carried out as described under Experimental Procedures. The conditions for competition experiments are given in the legend to Fig. 2. The displacement curves fitted either a one-site (●, ○, △) or two-site (▲) model.

for 75% of the total number of cerebral binding sites (Fig. 4 and Table 1). These findings indicate that the differential affinities of cerebral and atrial mAChRs for pirenzepine depended on the phosphatidylcholine to cholesteryl hemisuccinate ratio.

The displacement by pirenzepine of [^{3}H]QNB binding of cerebral receptors that were inserted into cholesteryl hemisuccinate and cholesteryl hemisuccinate/phosphatidylcholine mixtures (80:20 and 50:50, w/w) fitted the one-site model equation (Figs. 3 and 4 and Table 1). It has not been determined whether cerebral H sites were selectively inserted in these mixtures or whether the difference between the K_d values of H sites and L sites was too small to be detected with the competitive binding assay.

The role of lipids was further examined by inserting mAChRs into a mixture of phosphatidylinositol, cholesteryl hemisuccinate, and phosphatidylcholine. At the cholesteryl hemisuccinate/phosphatidylcholine/phosphatidylinositol ratio of 4:48:48 (w/w), the K_d of pirenzepine for atrial mAChRs (5.2 μM) was 61-fold higher than that of cerebral H sites (85 nM), which accounted for 73% of the total number of binding sites (Fig. 5 and Table 1).

Discussion

Purified mAChRs recovered their original membrane affinities for both pirenzepine and [^{3}H]QNB upon insertion into PrBCM-treated membrane preparations (Fig. 2 and Table 1). This finding suggests that the affinities of mAChRs for these antagonists are modulated by the interaction between the receptor molecules and membrane components, which are present in both cerebral and atrial membranes.

A preparation of purified cerebral mAChRs similar to the one used here was reported to contain both the M1 and M2 subtypes, as judged from the results of peptide analysis of purified preparations and cloning and expression of their cDNAs (4, 5, 8). The presence of both H sites and L sites for pirenzepine was not detected in the purified cerebral prepara-

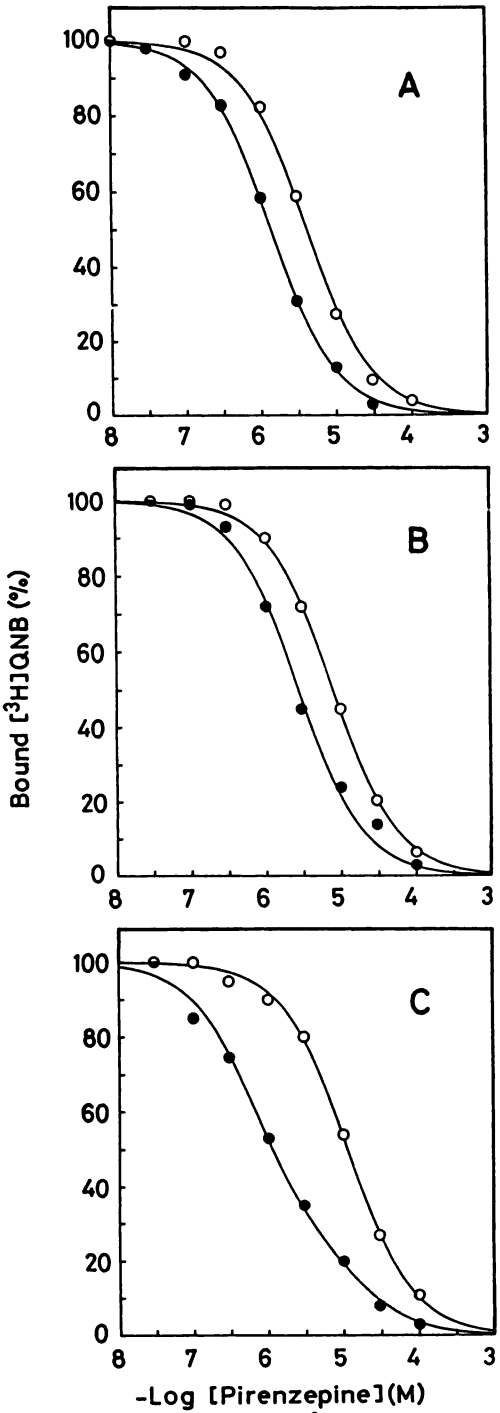

Fig. 4. Displacement by pirenzepine of [^{3}H]QNB binding of mAChRs purified from cerebrum (●) or from atrium (○) and then inserted into cholesteryl hemisuccinate/phosphatidylcholine mixtures, 50:50 (A), 80:20 (B), or 20:80 (C) (w/w). The experimental conditions were and data analysis was performed as in the legend to Fig. 2. The displacement curves fitted either a one-site [A, B, C (○)] or two-site [C (●)] model. The K_d values and proportions of H sites and L sites are given in Table 1.

tion in digitonin buffer solution (19, 26), but they became detectable after insertion of the purified receptors into PrBCM-treated membranes (Fig. 2 and Table 1). The affinities of cerebral L sites and of atrial receptors for pirenzepine are similar to each other (Table 1), suggesting that the cerebral L sites correspond to the M2 subtype.

A third and fourth mAChR subtypes, which have been found

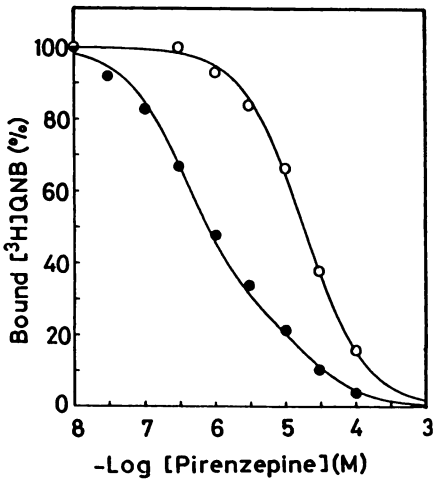

Fig. 5. Displacement by pirenzepine of [^{3}H]QNB binding of mAChRs purified from cerebrum (●) or atrium (○) and then inserted into a cholesteryl hemisuccinate/phosphatidylcholine/phosphatidylinositol (4:48:4, w/w) mixture. The experimental conditions were and data analysis was performed as in the legend to Fig. 2. The displacement curves fitted either a one-site (○) or two-site (●) model. The K_d values and proportions of H sites and L sites are given in Table 1.

in rat (4), human (5), and (the former) pig (7), were reported to bind pirenzepine with a 2- to 10-fold lower affinity than that of the M1 subtype. These observations imply that the pharmacologically defined M1 subtype might correspond to multiple mAChRs. The mRNAs encoding the four subtypes have been found in porcine cerebral cortex (28). A fifth subtype has been reported in human and rat (11), but it is not known in which tissue(s) it is expressed. The presence of any of the recently identified subtypes in the cerebral membrane preparation used in this study has yet to be determined. However, mAChR I seems to be the major component of the purified cerebral mAChR preparation, because the amino acid sequences of the major peptides obtained from partial hydrolysates of the preparation are included in the sequences of either mAChR I or mAChR II, but not in the sequences of the other subtypes, and the yield of peptides from mAChR I was much higher than that of those from mAChR II (4, 5).

The characteristics of the binding of agonists and nondiscriminatory antagonists to mAChRs in membrane preparations have been reported to change on alteration of the membrane lipid composition (see, for example, Refs. 29–31), suggesting that the lipid composition of the membrane affects the affinities of mAChRs for ligands. However, the relevant lipids might interact directly with the receptors and/or with other membrane proteins (such as G proteins), which in turn affects the binding properties of the receptors. In this study, insertion of purified mAChRs into lipid mixtures of defined compositions allowed the direct examination of the effect of the mAChR-lipid interaction on the affinities of receptors for antagonists.

The results in Fig. 3 and Table 1 suggest that the direct interaction between purified mAChRs and cholesteryl hemisuccinate results in high affinity of receptors for specific ligands. Cholesterol could not be used instead of cholesteryl hemisuccinate (a water-soluble derivative of cholesterol) under these experimental conditions because of its low solubility in water. It has been reported that the content of either cholesterol or cholesteryl hemisuccinate in membranes or reconstituted phospholipid vesicles affects the binding properties of several

G protein-coupled receptors, such as mAChRs (31), β-adrenergic receptors (32), and serotonin receptors (33). Considering that the amino acid sequences of the putative transmembrane domains of G protein-coupled receptors are similar to each other (34, 35), it is likely that the members of this receptor family have similar lipid-binding domains. Thus, the results presented here suggest the possibility that the effect of cholesterol on the affinities for ligands of G protein-coupled receptors is at least in part due to the direct receptor-cholesterol interaction.

When atrial and cerebral receptors were inserted into cholesteryl hemisuccinate/phosphatidylcholine mixtures, their affinities for [^{3}H]QNB were similar to each other at any given cholesteryl hemisuccinate/phosphatidylcholine ratio. In contrast, the affinity of atrial receptors for pirenzepine was 3- to 19-fold lower than that of cerebral H sites, depending on the cholesteryl hemisuccinate/phosphatidylcholine ratio (Fig. 4 and Table 1), indicating that the differential affinities of atrial and cerebral mAChRs for pirenzepine are affected by the lipid to lipid ratio. This effect might result either from changes in physical properties of the lipid environment due to the interaction of cholesteryl hemisuccinate with phosphatidylcholine or from competition between cholesteryl hemisuccinate and phosphatidylcholine for lipid binding sites on the receptor molecules. These two possibilities are not mutually exclusive. When phosphatidylinositol was added, with the cholesteryl hemisuccinate/phosphatidylcholine/phosphatidylinositol ratio of 4:48:48 (w/w), the affinity of cerebral H sites for pirenzepine was 61-fold higher than that of atrial receptors (Fig. 5 and Table 1), which is comparable to the value observed in membrane preparations. These findings, taken together, strongly suggest that the differential affinities of mAChR subtypes for pirenzepine are modulated by the lipid environment, and it is tempting to speculate that the lipid-binding domains of these molecules are involved in their pharmacological heterogeneity. The relevant lipids that interact with the receptors *in situ* remain to be identified.

In conclusion, the present results suggest that (a) purified mAChRs from porcine cerebrum and atrium recover their original affinities for [^{3}H]QNB and pirenzepine upon interaction with factors that are present in both cerebral and atrial membrane preparations; (b) the direct interaction between purified mAChRs and cholesteryl hemisuccinate results in an affinity of receptors for specific antagonists that is comparable to the membrane values; and (c) the affinities of mAChRs for antagonists, in particular the differential affinities of the M1 and M2 subtypes for pirenzepine, are modulated by the lipid environment.

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