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Lipid modulation of nicotinic acetylcholine receptor function: the role of membrane lipid composition and fluidity

Carl Sunshine, Mark G. McNamee *

Section of Molecular and Cellular Biology, University of California, Davis, CA 954616, USA

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Abstract

The effects of membrane lipid composition and fluidity on AChR ion channel function were studied after reconstituting the receptor with sphingomyelin, phosphatidylcholines with different degrees of unsaturation, or different neutral lipids. AChR ion flux activity was shown to be retained in some membranes of both high and low fluidity, as measured by the steady-state anisotropy of the membrane probes diphenylhexatriene and trimethylammonium diphenylhexatriene. The results suggest that lipid composition is more important than bulk membrane fluidity in determining AChR ion channel function.

Key words: Nicotinic acetylcholine receptor; Membrane fluidity; Lipid composition; Ion channel function

1. Introduction

Membrane proteins exist in a fluid lipid bilayer environment and protein conformational changes may be sensitive to membrane fluidity or some other bulk property of the membrane. Membrane fluidity is a semi-quantitative term encompassing all lipid molecular motions throughout the bilayer, such as lateral and rotational motions, *trans-gauche* isomerizations of the acyl chains and anisotropic motions [1]. One example where membrane order or fluidity is important to protein function is rhodopsin [2], where a photolytic conformational transition was shown to be sensitive to cholesterol concentration in reconstituted membranes. The motional order of the lipid acyl chains in most cell membranes, under physiological conditions, is reduced by the presence of cholesterol [3] and Mitchell et al. [2] showed that an increase in cholesterol concentration progressively reduced the equilibrium constant for the rhodopsin allosteric transition. This effect was paralleled by a corresponding increase in the dynamic fluorescence anisotropy of diphenylhexatriene (DPH), a measure of membrane fluidity.

In contrast, East et al. [4] showed that fluidity, as measured by the EPR order parameter of 5-doxylstearic acid, was not an important regulator of the activity of the Ca/Mg-ATPase from sarcoplasmic reticulum in reconstituted membranes containing different unsaturated phosphatidylcholines. Their results showed that lipid composition was a much more important determinant of activity than bulk membrane fluidity. However, the results of Bigelow et al. [5] strongly suggested that the rotational mobility of the Ca/Mg-ATPase was essential for enzyme activity. Protein rotational mobility has been predicted to be proportional to the lipid fluidity and the protein surface area in contact with the lipids [6]. Bigelow et al. determined, using EPR techniques, that both the lipid fluidity and ATPase rotational mobility increased with an increase in temperature, resulting in linear Arrhenius plots. The activation energy for ATPase rotation was found to be the same as that for enzyme activity above 20°C.

The nicotinic acetylcholine receptor from *Torpedo californica* electric organ (AChR) has also been widely

* Corresponding author. Fax: +1 (916) 7523085.

Abbreviations: AChR, nicotinic acetylcholine receptor; Ca/Mg-ATPase, calcium and magnesium-dependent adenosine triphosphatase; Carb, carbamylcholine chloride; CH, cholesterol; DEPC, dielaidoylphosphatidylcholine; DELPC, dilinolenoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DPH, diphenylhexatriene; EPR, electron paramagnetic resonance; Mops, 3-(*N*-morpholino)propanesulfonic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PS, phosphatidylserine; TMA-DPH, trimethylammonium diphenylhexatriene; TO, α-tocopherol.

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used as a model system for studying the effects of lipid composition and membrane fluidity on protein structure and function. The AChR, a pentameric integral membrane protein critical for cholinergic synaptic transmission [7], has been purified and reconstituted into defined lipid environments [8]. Three aspects of AChR function have been studied in detail: the binding of agonists and antagonists, the activation of a cation channel upon agonist binding, and the desensitization process in which ion conduction is blocked in the prolonged presence of agonist. Both channel activation and the rate of desensitization have been shown to be sensitive to the lipid composition in reconstituted membranes [9].

Previous results suggested that the AChR ion channel-gating conformational transition required an optimal membrane fluidity, in addition to the requirement for cholesterol and negatively charged phospholipids. Fong and McNamee [1] showed that the AChR ion flux activity occurred, in a limited number of membrane samples, within a narrow window of the order parameter, determined by EPR spectra of 5-doxylstearic acid. The experiments here were designed to extend the studies of Fong and McNamee by including a greater number of fluidity and ion flux measurements on AChR membranes in the liquid-crystalline and solid phases. The fluidity of the AChR membranes was varied by reconstituting with sphingomyelin, phosphatidylcholines with different degrees of unsaturation, or different neutral lipids. In addition, all reconstituted AChR membranes contained negatively charged lipids.

Results presented here show that AChR ion channel activity can be retained in some membranes of both high and low fluidity, as measured by the steady-state fluorescence anisotropy of the membrane probes DPH and trimethylammonium diphenylhexatriene (TMA-DPH). The results suggest that lipid *composition* is more important than bulk membrane fluidity in determining AChR ion channel function.

2. Materials and methods

Materials. Chloroform solutions of all synthetic phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol was obtained from Calbiochem (LaJolla, CA). Dowex 50W-X8 cation exchange resin and cholic acid were obtained from Sigma (St. Louis, MO). $^{86}Rb^+$ (10 mCi) was obtained from New England Nuclear (Boston, MA). DPH and TMA-DPH were obtained from Molecular Probes (Eugene, OR).

Acetylcholine receptor purification and reconstitution. The acetylcholine receptor protein was purified from frozen *T. californica* electric organ by affinity chromatography, as described by Ochoa et al. [10] and then dialyzed to remove the cholate detergent. The receptor was used immediately or frozen in liquid nitrogen and then thawed immediately before use.

Choroform solutions of phospholipids and cholesterol were mixed in the amounts necessary to obtain a final lipid/protein mole ratio of 10000:1 and a mole ratio of 60:20:20 for phosphatidylcholine (or sphingomyelin)/dioleoylphosphatidic acid/cholesterol or 50:10:20:20 for dilinolenoylphosphatidylcholine or distearoylphosphatidylcholine or sphingomyelin/dioleoylphosphatidylcholine/dioleoylphosphatidic acid/cholesterol. The lipids were then dried down under nitrogen and lyophilized. Subsequently, the lipids were sonicated in the presence of 6% (w/v) cholate for 3 min to give homogeneous suspensions. Only lipid mixtures containing DSPC were not homogeneous after sonication and formed a milky precipitate. Purified AChR was mixed with the lipid suspension in a ratio of 2:1 (v/v). The final cholate and protein concentrations were 2% and 1 mg/ml, respectively. The solubilized lipid-protein micellar suspension was then extensively dialyzed against Buffer A (100 mM NaCl, 10 mM Mops, 0.1 mM EDTA, 0.02% NaN_3, pH 7.4) to remove the cholate and allow the formation of vesicles.

Ion flux assays. Carbamylcholine-stimulated influx of $^{86}Rb^+$ into AChR-containing vesicles was measured according to Fong and McNamee [4]. Flux assays were performed at 4°C in triplicate on each sample. Typically, ion flux was allowed to occur for 30 s in the presence or absence of activating ligand prior to separation of the external solution from the vesicles by cation exchange chromatography. 15 μl of 0.5–1 mCi/ml $^{86}Rb^+$, containing 4.33 mM Carb or Buffer A only, was added to 50 μl of membranes and flux was allowed to occur for 30 s at 4°C. A 50 μl aliquot was then applied to a 2 ml Dowex 50W-X8 column and eluted with 3 ml of 175 mM sucrose. The eluate was counted in a Packard Tri-Carb 1500 Liquid Scintillation Analyzer, using a 0–1770 KeV window, without added scintillant. The total internal volume of the vesicles was determined by allowing influx to occur for at least 48 h.

Fluorescence measurements. Samples were prepared for fluorescence measurements by incubating 100 μl of membranes, plus 3 ml Buffer A, with 20 μl of 1 mM DPH or TMA-DPH at 37°C for at least 2 h. DPH was dried down under N_2 prior to the addition of membranes and Buffer A. The TMA-DPH stock solution was prepared in dimethylsulfoxide, while the DPH stock solution was prepared in tetrahydrofuran.

All measurements of fluorescence polarization of DPH and TMA-DPH were taken on an SLM-4800 spectrofluorimeter configured in the 'T' type format, with excitation and emission wavelengths of 360 and 440 nm, respectively. Five values of each component of fluorescence intensity, measured with parallel and per-

pendicularly oriented polarizers, were obtained in order for the on-line computer to calculate the polarization (P). At least three values of the polarization were obtained for each membrane sample. The steady-state anisotropy of fluorescence has been calculated by using the equation: $r_s = 2P/(3 - P)$ [11].

3. Results

Ion flux activity of vesicles in different lipid compositions

Sealed vesicles are required to measure the ion flux activity of the AChR. Therefore, receptor samples with a lipid to protein mole ratio of 10000:1 were reconstituted to allow formation of appropriate vesicles. Table 1 lists the results of the rubidium ion influx assays for AChR vesicle samples containing different zwitterionic or neutral lipids. Each $^{86}Rb^+$ influx reaction was allowed to occur for 30 s at 4°C in the presence or absence of Carb. Because the $^{86}Rb^+$ influx is affected by vesicle size, a parameter termed 'response' was calculated by normalizing the apparent internal volume (IV) of trapped $^{86}Rb^+$, due to Carb activation of ion channels, to the equilibrium internal volume (EIV). Based on previous experience with reconstituted membranes [9], the AChR ion flux response for dioleoylphosphatidylcholine/dioleoylphosphatidic acid (DOPC/DOPA) vesicles was used as a standard for basal or low activity, while the flux response of DOPC/DOPA/CH vesicles was used as a standard for optimal or high activity.

Table 1 and the legend contain the compositions, full names, and abbreviations for all the lipids used. Comparison of membrane samples reconstituted with unsaturated phosphatidylcholines showed that the ion flux responses decreased as the number of double bonds increased. Vesicles containing a fully saturated PC, DSPC/DOPA/CH, gave a low activity ion flux response, compared to DOPC/DOPA/CH. The ion flux response for SPH/DOPA/CH vesicles cannot be directly compared to the other membranes, since the EIV for this composition was very low. The presence of 10 mole% DOPC in AChR vesicle samples which originally gave low activity flux responses increased the activity at least two-fold. Thus, DSPC/DOPC, SPH/DOPC and DELPC/DOPC membranes showed active AChR ion channels. However, the ion flux activity of the DELPC/DOPC membranes was still close to the basal level response.

As an alternative method to alter the fluidity and composition of AChR membranes, squalene, a neutral lipid unrelated in structure to cholesterol, was used to reconstitute vesicles with a DOPC/DOPA/SQU composition. These membranes were previously shown to support an optimal ion flux response [9]. Membranes containing squalene instead of cholesterol showed a relatively high ion flux response, compared to the basal level.

Fluorescence anisotropy for vesicles with different lipid compositions

Table 2 gives values for the fluorescence anisotropy, determined at 6°C, of DPH and TMA-DPH for each AChR vesicle sample whose ion flux activity was determined. DPH and TMA-DPH probe different membrane domains [12]. DPH is located within the hydrophobic membrane core, aligned with the phospholipid acyl chains, while the trimethylammonium head of TMA-DPH interacts with the phospholipid polar headgroups. The values for the fluorescence anisotropy for each AChR membrane sample were, in general, higher for TMA-DPH than DPH. A major exception were membranes containing the solid phase lipids DSPC and SPH.

Table 1
Ion flux responses of AChR-containing membranes of varying lipid composition

Composition [a]	+Carb (CPM)	−Carb (CPM)	EIV (CPM)	IV (μl/ml)	EIV (μl/ml)	Response [b] (×10²)
DOPC/DOPA (80:20)	3218	1194	14994	0.31	2.31	13 ± 1
DOPC/DOPA/CH (60:20:20)	13808	608	24148	2.03	3.72	55 ± 1
DLPC/DOPA/CH (60:20:20)	6993	457	15129	1.01	2.33	43 ± 1
DELPC/DOPA/CH (60:20:20)	1981	349	12362	0.25	1.90	13 ± 1
DSPC/DOPA/CH (60:20:20)	2470	889	7478	0.24	1.15	21 ± 1
SPH/DOPA/CH (60:20:20)	704	233	2209	0.07	0.34	21 ± 2
DELPC/DOPC/DOPA/CH (50:10:20:20)	4074	406	13366	0.56	2.06	27 ± 1
DSPC/DOPC/DOPA/CH (50:10:20:20)	3599	736	7047	0.44	1.09	41 ± 3
SPH/DOPC/DOPA/CH (50:10:20:20)	4747	609	7115	0.64	1.10	58 ± 6
DOPC/DOPA/SQU (60:20:20)	30761	9363	55155	3.30	8.49	39 ± 3

[a] The lipid mole ratio for each composition is given in parentheses. Abbreviations (the number of double bonds in the phospholipid acyl chains are given in parentheses): CH, cholesterol; DOPC, dioleoylphosphatidylcholine (18:1); DOPA, dioleoylphosphatidic acid (18:1), DLPC, dilinoleoylphosphatidylcholine (18:2); DELPC, dilinolenoylphosphatidylcholine (18:3), DSPC, distearoylphosphatidylcholine (18:0); SPH, sphingomyelin (from bovine brain, rich in steric and nervonic acid); SQU, squalene.

[b] Response = IV/EIV. Errors are given as the standard deviation of the mean for triplicate samples.

The basis for the use of the fluorescence anisotropy as a measure of the fluidity of the membranes is that the rotational mobility of the probes is negatively correlated with the anisotropy. Therefore, probe molecules within a relatively solid membrane will be much more immobilized, and consequently, have a larger value of the anisotropy, than probe molecules within a fluid membrane.

The most apparent trend in Table 2 is the increase in the anisotropy with a decrease in the number of double bonds in the PC molecule reconstituted with the AChR. The largest change in anisotropy for DPH or TMA-DPH, for the PC series, occurs in AChR membrane samples reconstituted with DSPC or DOPC. The AChR sample containing DSPC (no DOPC) had an anisotropy for DPH of 0.356, close to the theoretical limit of 0.4 for complete immobilization of the probe. This suggests that these membranes containing DSPC are in the solid phase. Membranes containing DSPC or SPH were highly comparable in anisotropy values and showed the highest anisotropy values in both the DPH and TMA-DPH series. By extrapolation, this suggests that membranes containing SPH are also in the solid phase. The presence of 10 mole% DOPC, in membranes containing SPH, DSPC or DELPC did not significantly change the anisotropy for the probes. This suggests that the fluidity of these membranes is not significantly altered by the presence of a small amount of DOPC. However, these membranes had a functional ion flux response. These results provide evidence that lipid composition is more important than bulk membrane fluidity in determining the activity of the AChR.

Table 2
Fluorescence anisotropy values for DPH and TMA-DPH at 6°C for membranes of varying lipid composition

Composition [a]	Anisotropy [b]	
	DPH	TMA-DPH
DOPC/DOPA (80:20)	0.167 ± 0.0002	0.2253 ± 0.0001
DOPC/DOPA/CH (60:20:20)	0.209 ± 0.001	0.2361 ± 0.0002
DLPC/DOPA/CH (60:20:20)	0.177 ± 0.001	0.2246 ± 0.0004
DELPC/DOPA/CH (60:20:20)	0.164 ± 0.001	0.2202 ± 0.0002
DSPC/DOPA/CH (60:20:20)	0.356 ± 0.001	0.2835 ± 0.0003
SPH/DOPA/CH (60:20:20)	0.351 ± 0.001	0.2590 ± 0.0005
DELPC/DOPC/DOPA/CH (50:10:20:20)	0.169 ± 0.001	0.2366 ± 0.0007
DSPC/DOPC/DOPA/CH (50:10:20:20)	0.344 ± 0.001	0.2628 ± 0.0003
SPH/DOPC/DOPA/CH (50:10:20:20)	0.335 ± 0.001	0.2370 ± 0.0001
DOPC/DOPA/SQU (60:20:20)	0.165 ± 0.001	0.2117 ± 0.0001

[a] The lipid mole ratio for each composition is given in parentheses. Abbreviations are given in the legend to Table 1.
[b] Errors are given as the standard deviation of the mean for at least triplicate measurements.

Table 3
Ion flux responses and corresponding fluorescence anisotropy values for DPH at 25°C for membranes of varying lipid composition

Composition [a]	Response ($\times 10^2$) [b]	Anisotropy [c]
DOPC/DOPS	1.2 ± 0.2	0.0961 ± 0.0007
DOPC/DOPA	8.1 ± 1	0.0970 ± 0.0008
DOPC/DOPS/CH	12.0 ± 1	0.1223 ± 0.0004
DOPC/DOPA/SQU	19.6 ± 1	0.0630 ± 0.0001
DOPC/DOPA/CH	27.3 ± 3	0.1240 ± 0.0004
DOPC/DOPA/AND	31.4 ± 3	0.0967 ± 0.0008
DOPC/DOPS/TOC	35.6 ± 2	0.1348 ± 0.0001
DOPC/DOPA/TOC	53.9 ± 8	0.1298 ± 0.0003

[a] Most of the vesicle samples contain PC, negatively charged lipid and neutral lipid in the mole ratio 60:20:20. Samples containing only PC and negatively charged lipid have a lipid mole ratio of 80:20. Abbreviations: AND, androstanol; DOPS, dioleoylphosphatidylserine; TOC, α-tocopherol. Other abbreviations are given in the legend to Table 1.
[b] Response = IV/EIV. Ion flux data was obtained from Ref. [9].
[c] Errors are given as the standard deviation of the mean for at least triplicate measurements.

The AChR membrane sample containing squalene showed values for the fluorescence anisotropy which were highly comparable to the values for samples containing DELPC (without DOPC). The squalene and DELPC samples showed the lowest anisotropy values within the DPH or TMA-DPH series This suggests that these membranes were the most fluid ones studied. It is important to note that the DELPC sample only showed basal ion flux activity, while the vesicle sample with squalene was fully functional. This further emphasizes the importance of lipid composition in supporting AChR ion flux activity.

Table 3 shows values of the fluorescence anisotopy for DPH, determined at 25°C, for several AChR membrane samples whose ion flux activities were previously determined [9]. All the samples listed in Table 3 showed functional ion channels, with the exception of DOPC/DOPA and DOPC/DOPS membranes, which showed basal level activity and no activity, respectively. Because the squalene vesicle sample, which showed a functional ion flux response, had an anisotropy which was lower than the DOPC/DOPA and DOPC/DOPS samples, there can be no correlation between the anisotropy values listed in Table 3 and the corresponding ion flux responses. Comparison of the anisotropy values of membrane samples containing DOPA or DOPS, which are reconstituted with the same neutral lipid or no neural lipid, showed no significant differences.

Table 4
Ion flux responses and corresponding DPH anisotropy values at 25°C for AChR membranes of varying sphingomyelin content

Composition [a] SPH/DOPC/DOPA/CH	Response ($\times 10^2$) [b]	Anisotropy [c]
0:60:20:20	54±1	0.1187±0.0004
5:55:20:20	57±1	0.1261±0.0002
10:50:20:20	52±3	0.1446±0.0004
30:30:20:20	63±2	0.1993±0.0004
50:10:20:20	58±6	0.2529±0.0002
55:5:20:20	59±1	0.2673±0.0002

[a] Values given represent lipid mole ratios. Abbreviations are given in the legend in Table 1.
[b] Response = IV/EIV.
[c] Errors are given as the standard deviation of the mean of triplicate samples.

The differences in anisotropy values for AChR membranes reconstituted with DOPA containing cholesterol, androstanol, squalene or tocopherol are not functionally relevant, since all these membrane samples have a functional ion flux response. However, these differences suggest that the fluidity of the membranes can be modulated, to a small extent, by reconstituting with different neutral lipids.

Table 4 shows the ion flux responses and the corresponding values for the fluorescence anisotropy of DPH at 25°C, for a series of AChR membranes containing increasing amounts of SPH, in the presence of DOPC/DOPA/CH. Table 4 shows that the anisotropy increases with an increase in the SPH content of the membranes. However, the ion flux responses measured for the entire series showed that these membranes were all functional. Furthermore, the responses did not significantly differ with increases in SPH content in the AChR membranes, strongly suggesting that the anisotropy is independent of the ion flux response.

4. Discussion

The work presented here was performed in an attempt to determine a relationship between the AChR ion flux response and membrane fluidity. To describe bulk membrane fluidity, we have used the steady-state anisotropy, which is determined by the rotational relaxation time and the lifetime of the fluorophores [11]. No consistent correlation between the ion flux response and the fluorescence anisotropy of DPH or TMA-DPH, two membrane probes which are commonly used in measurements of membrane order, was found. The results reported herein strongly suggest that lipid composition is more important than bulk membrane fluidity in determining the function of the AChR.

The results of Fong and McNamee [1] suggested that the ion channel-gating conformational transition of the AChR required an optimal bulk membrane fluidity, in addition to the requirement for cholesterol and negatively charged lipids. They observed ion channel function only within a small range of the EPR order parameter (0.75–0.83 at 0°C) for 5-doxylstearic acid, determined on a limited number of defined lipid compositions. Fong and McNamee were able to change the fluidity of the AChR membranes by altering the chain length or type of double bond (*cis* or *trans*), or the number of double bonds of the PC used in the reconstitution. The work reported here has extended their study by including fluidity and ion flux measurements on a greater number of lipid compositions in the liquid-crystalline and solid phases. The fluidity and composition of the AChR membranes was varied by reconstituting with sphingomyelin, phosphatidylcholines with different degrees of unsaturation, different neutral lipids or the inclusion of small amounts of DOPC into solid or fluid phase compositions.

The ion flux responses for the series of compositions reconstituted with PCs containing from zero to three double bonds are consistent with the optimal fluidity hypothesis of Fong and McNamee. However, the effects of lipid composition (for example, the inclusion of small amounts of DOPC into solid phase lipids or the replacement of cholesterol by squalene) were shown to be more important than bulk membrane fluidity, since no correlation between the fluorescence anisotropy of DPH or TMA-DPH and AChR ion flux activity was observed.

Similarly, East et al. [4] have shown that there was no correlation between the enzymatic activity of the reconstituted Ca/Mg-ATPase and the EPR order parameter (0.5–0.6 at 37°C), for membranes containing different types of unsaturated phosphatidylcholines. However, the optimal fluidity hypothesis of Fong and McNamee may stand in a modified form. An optimal fluidity of the lipids directly surrounding the AChR could be necessary for the function of the receptor. The correlation between the rotational mobility of the Ca/Mg-ATPase and enzyme activity [5] could suggest that the fluidity of the lipids surrounding the ATPase can control protein function.

One physical mechanism to explain why an optimal fluidity of the annular lipids might be necessary for AChR ion channel function is the matching of the dynamic structure of the phospholipid acyl chains to the structure of the protein. In this speculative scheme, the acyl chains which can most adapt to the membrane exposed surface of the AChR would allow for optimal ion channel function. The increase in the ion flux response with a decrease in the number of double bonds in the PC molecule reconstituted with the AChR, and the apparent exception of a fully saturated PC, DSPC to this trend is consistent with this hypothesis. Furthermore, the increase in the ion flux response when DELPC, SPH or DSPC membranes are supple-

mented with 10 mole% DOPC is consistent with the idea that the acyl chains of DOPC optimally match the structure of the AChR. The lipid composition effects on the ion channel function of the AChR may reflect the requirement for an optimal fluidity of the annular lipids.

Data consistent with different motional or fluidity properties of the annular and bulk phase lipids was obtained using the EPR spectra of fatty acid and sterol spin probes in native and cholesterol-enriched microsomal membranes. Castuma et al. [13] showed that the EPR spectra contained two components, one characteristic of bulk bilayer lipids, and the second characteristic of motionally restricted lipids interacting with membrane proteins. Cholesterol modulated the motional properties of the two lipid domains in opposite ways. Cholesterol enrichment decreased the mobility and increased the order of the bulk lipids, and increased the mobility and decreased the order of the motionally restricted component.

Recent studies on glycophorin are consistent with the hypothesis that glycophorin-cholesterol interactions can lead to the formation of sterol-enriched lipid domains in the vicinity of the protein. Glycophorin has been shown to immobilize a cholestanol spin label in reconstituted DMPC vesicles [14], suggesting a strong interaction of the protein with the sterol. Tampe et al. [15] were able to construct a phase diagram of DMPC/cholesterol membranes in the presence and absence of glycophorin, using differential scanning calorimetry. Glycophorin at low mole ratios increased the amount of lipid in the liquid-ordered phase. This was interpreted as an enrichment of cholesterol in the vicinity of the protein. Tampe et al. [15] gave a direct demonstration of sterol-enriched domain formation using EPR on a spin-labeled cholesterol analogue. There was a large increase in the spin–spin interaction of the labeled sterol in the presence of glycophorin.

Thus, the lipids which interact with membrane proteins may have different fluidity, phase and composition properties than the bulk lipids. Narayanaswami and McNamee [16] have provided data consistent with the hypothesis of a fluid layer of lipids surrounding the AChR protein in reconstituted membranes, using fluorescence quenching of tryptophan by *cis*- and *trans*-parinaric acid. In membranes containing DEPC, *cis*-parinaric acid quenched the AChR fluorescence more effectively than *trans*-parinaric acid, at a temperature below the phase transition. At a temperature above the phase transition, both forms of parinaric acid were equally effective in quenching AChR fluorescence. Since *trans*-parinaric acid preferentially partitions into solid phase lipids, the results suggested that there was a fluid layer of lipids surrounding the AChR.

A possible interpretation of the results of Narayanaswami and McNamee [16] is that the interaction of lipids with the AChR induces the formation of a fluid domain of lipids surrounding the protein. This would also be expected to apply to the multi-component lipid membranes used in this study. However, it is also possible that the AChR may preferentially partition into a more fluid lipid domain due to a lateral phase separation, in the solid phase lipid compositions which were shown here to support ion channel activity. The AChR may only show an optimal ion flux response in a fluid lipid domain. Techniques which have been useful for the dectection of lipid domains in membranes, such as dynamic fluorescence anisotropy [17], probably could not distinguish protein-induced domain formation from lateral phase separation. However, it may be possible to extend the present study by contructing phase diagrams of the membranes, using differential scanning calorimetry, to determine if the presence of the AChR induces major changes in the phase equilibria of the lipids.

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6. References

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