Insertion of the amyloid precursor protein into lipid monolayers: effects of cholesterol and apolipoprotein E

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APP (amyloid precursor protein), together with Chol (cholesterol) and ApoE (apolipoprotein E), has been linked to Alzheimer's disease. We have examined the hypothesis that interaction of APP with the lipid membranes is modulated by Chol and ApoE. Insertion of APP into lipid monolayers was first evidenced as an increase in the surface pressure. APP injected into a subphase induced a substantial increase in the surface pressure of monolayers prepared from PC (L- α -phosphatidylcholine), Chol, SPM (sphingomyelin) and PS (L- α -phosphatidylserine), the major lipids present in the plasma membranes of brain cells. At a given initial pressure, the insertion of APP into expanded monolayers is higher than that in condensed monolayers, in the order Chol > PC > SPM > PS. The membrane insertion capacity of APP was also measured from surface pressure versus area (π -A) isotherms of APP-lipid monolayers. The increase in the mean

area per molecule in protein-lipid monolayers, in the order PC > Chol > PS > SPM, provides further evidence for protein-lipid interactions. These interactions occurred at optimum salt levels and optimum pH values close to physiological conditions (150 mM NaCl and pH 7.4). In addition, ApoE4 affected the insertion of APP into lipid films. APP-ApoE complexes showed a decreased ability to penetrate lipid monolayers at a constant area. APP-ApoE complexes expanded the π -A isotherm of a Chol monolayer to a lesser extent than APP alone. These experiments demonstrate the roles of Chol and ApoE in the modulation of membrane insertion of APP.

Key words: Alzheimer's disease, amyloid precursor protein, apolipoprotein E (ApoE), lipid-protein interaction.

INTRODUCTION

AD (Alzheimer's disease) is a progressive neurodegenerative disorder characterized by the occurrence of senile plaques and neurofibrillary tangles throughout the brain cortex [1,2]. The core component of the extracellular fibrillar deposits is the A β (amyloid β -peptide), which is the proteolytic product of the larger transmembrane APP (amyloid precursor protein). This proteolytic cleavage is mediated by β - and γ -secretases. APP can also be processed through a non-amyloidogenic pathway in which α -secretase cleaves the protein to release sAPP (soluble APP) [3]. A β is produced from the proteolytic cleavage of APP in the endoplasmic reticulum to generate A β 42 and in the *trans*-Golgi network to generate A β 40 [4,5]. It has also been suggested that A β 40/42 may be generated at the plasma-membrane surface. The presence of A β in distinct compartments and the hypothesis that lipid association is important for both neurotoxicity and fibrillogenesis suggest that the lipid composition and characteristics of these compartments may play vital roles in the disease processes [6]. Furthermore, it was recently reported that formation of A β fibrils is highly sensitive to lipid environments and more readily occurred in ganglioside raft-like membranes composed of Chol (cholesterol) and SPM (sphingomyelin) [7,8]. Lipid rafts are regions of the plasma membrane enriched in Chol and glycosphingolipids, and they have been proposed to be sites of proteolytic processing of APP [9,10]. Recent studies revealed that APP processing is modulated by lipid rafts [11,12]. Although aggregated A β is one of the main components, several other proteins are also associated with senile plaques, including APP itself, α 1-antichymotrypsin, glycosaminoglycans and, most notably, ApoE (apolipoprotein E) [13,14]. The intriguing relationship

between AD and ApoE is corroborated by the finding that APP proteolysis is affected in patients carrying the ϵ 4 allele of ApoE. Several studies have demonstrated that synthetic A β , as well as APP, binds *in vitro* to ApoE [15–19]. Binding of ApoE to APP suggested a mechanism in which ApoE isoforms may modulate APP degradation, thus modulating A β formation and aggregation and, consequently, the risk for AD [16–20].

In the present study, we describe the membrane insertion property of APP. Using penetration into lipid monolayers at liquid/air interfaces, we have shown that APP is capable of inserting itself into a lipid monolayer, which depends on the ratio of Chol to PC (L- α -phosphatidylcholine) or SPM and on the packing density of lipid monolayers. The mode of association of APP with ApoE on a lipid surface may be important for ApoE to play its role in lipid metabolism or may be important for APP metabolism [20]. Therefore we investigated whether ApoE is capable of interacting with APP in a lipid environment. The binding of ApoE to APP was assessed by the monolayer technique to study the influence of ApoE on the behaviour of APP at the air/lipid interface. The results presented in this study clearly show that ApoE binds APP in a manner that decreases the interaction of APP with the Chol monolayer or with the PC monolayer. The APP-ApoE complexes produced *in vitro* appear to be stabilized by strongly resistant intermolecular interactions even in the presence of lipids.

MATERIALS AND METHODS

Materials

APP was purified from porcine brains as described previously [21]. The procedure yields homogeneous preparations as judged

Abbreviations used: AD, Alzheimer's disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; A β , amyloid β -peptide; Chol, cholesterol; LE phase, liquid-expanded phase; PC, L- α -phosphatidylcholine; PS, L- α -phosphatidylserine; SPM, sphingomyelin.

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by SDS/PAGE [22]. The protein concentration was determined by the Bradford dye-binding assay [23] using BSA as a standard. PC from egg yolk, PS (L- α -phosphatidylserine), Chol and SPM from bovine brain were purchased from Sigma (St. Louis, MO, U.S.A.). Human recombinant ApoE4 was purchased from Calbiochem (Merck, Darmstadt, Germany). All other reagents were of analytical grade.

Monolayer experiments

The monolayer surface pressure π , defined as the change in surface tension after spreading a monolayer on a water surface, was measured with a Wilhelmy trough and an R&K tensiometer (Wiesbaden, Germany) as described previously [24]. A filter paper (Whatmann no. 1) was employed as the Wilhelmy plate. During the measurement, the subphase was continuously stirred with a magnetic bar. The temperature was controlled at 21 °C. To measure the interaction of APP with the lipid monolayer, a circular Teflon trough with a volume of 5 ml and a surface area of 4.9 cm² was used. The trough was filled with a buffer composed of 20 mM Tris/HCl (pH 7.4) and 150 mM NaCl. A monomolecular lipid film was formed by carefully spreading the lipid solution (0.5 mM) with a Hamilton syringe on the buffer surface to the desired initial surface pressure π_i , allowing the solvent to evaporate for approx. 20 min. APP (4.5 nM) was injected into the buffer subphase through a side sample hole. The increase in surface pressure $\Delta\pi$ was monitored until it reached a maximal value, usually within 3 h. For each sample, the values of $\Delta\pi$ as a function of various π_i values were obtained. The plot of $\Delta\pi$ versus π_i yields a straight line with a negative slope, which intersects the abscissa axis at a limiting surface pressure. This limiting surface pressure is defined as the critical insertion pressure π_c of APP for the corresponding lipid monolayer.

The π - A isotherms were obtained using a rectangular Teflon trough (26 cm $\times$ 2.6 cm; surface area 68 cm²) with two movable Teflon barriers. Lipid monolayers were formed on the clean air/water interface by spreading known amounts of lipid dissolved in chloroform. The surface area was reduced at the rate of 2 cm² $\cdot$ min⁻¹ with the Teflon barriers, and the change in π versus A was recorded after 20 min to allow solvent evaporation. After the maximum compression was reached (usually 40 mN/m), the lipid monolayer was expanded back to the original area. The extrapolated molecular area at $\pi=0$ was defined as the limited molecular area A_0 and calculated from π - A isotherms. After an appropriate waiting period to allow monolayer relaxation, a known amount of APP was carefully spread on the preformed lipid monolayer. After a further 30 min lag period, compression was performed at the rate of 2 cm² $\cdot$ min⁻¹ and the lipid-protein (π - A) isotherm was recorded. For binding experiments with APP and ApoE, 45 pmol of APP was mixed with 45 pmol of ApoE and, after a short incubation period, the solution was spread over the lipid/water interface. The π value was recorded and plotted as a function of the molecular area of the lipid molecules.

RESULTS

Insertion of APP within PC, Chol, SPM and their mixed monolayers

The interaction of APP with lipid monolayers was analysed using the Langmuir balance technology. In these experiments, APP was added at a constant area to the aqueous subphase underneath a monomolecular lipid film, and the resulting interaction was measured as the increase in the surface pressure of the film. The lipid specificity of the protein insertion was first investigated by injecting APP (final concentration of 4.5 nM) under different

Figure 1 Insertion profile of APP into lipid monolayers

π - t curves of the monolayer insertion of APP, after injection, underneath the monomolecular film of 4.5 nM Chol (thick solid line), PC (.....), SPM (---) or PS (—), which were spread at an initial surface pressure of 10 mN/m.

types of lipid monolayers (PC, Chol, PS and SPM monolayers). The values obtained for $\pi_i = 10$ mN/m are presented in Figure 1. APP insertion provoked an immediate increase in the surface pressure. These results suggest a very fast diffusion and incorporation process of APP into the lipid film. The rate of insertion of the protein at the lipid/water interface increased considerably in comparison with the surface activity of the protein at the air/water interface. In the absence of lipid, for a protein concentration of 4.5 nM, the surface pressure started to increase after 50 min, as reported previously [24]. The kinetics and extent of surface pressure increase were very sensitive to the composition of the lipid monolayer (Figure 1). At 4.5 nM, APP induced a 14 mN/m increase in the surface pressure of the PC monolayer, i.e. a 140 % increase in the initial surface pressure ($\pi_i = 10$ mN/m). A greater expansion of the film was measured when APP was added to a Chol monolayer since the surface pressure increased by 320 %. At the same concentration of APP in the subphase and during the same time scale, the $\Delta\pi$ variation observed with SPM was weaker (10 mN/m) compared with the experiments described above (100 % increase in the π_i). A weaker increase in $\Delta\pi$ was observed previously when APP was injected under a PS monolayer (Figure 1) [24]. To assess further the lipid specificity of the penetration process, monolayers consisting of the same lipids were prepared at various initial pressures. The increase in surface pressure $\Delta\pi$ caused by the penetration of APP into the monolayer was measured as a function of the initial pressure of the monolayer after the equilibrium had been reached (Figure 2). The values of critical pressure π_c for APP insertion into a Chol monolayer or an SPM monolayer (i.e. the theoretical value of π_i extrapolated for $\Delta\pi_{\max} = 0$ mN/m) were 32 and 28 mN/m respectively. The numerical values of π_c for PC and PS monolayers have been determined previously to be 23 and 11 mN/m respectively [24]. These results indicate that APP interacts specifically with the monolayers of Chol, SPM and PC and, to a lesser extent, with the PS monolayer. However, even the π_c values for the Chol and SPM monolayers are closely related and the $\Delta\pi$ values were always lower for the SPM monolayers. The importance of the Chol content of the monolayer in protein insertion was confirmed by performing experiments with PC or SPM monolayers in the presence of different molar fractions of Chol (Figure 3). Clearly, $\Delta\pi$ rapidly increased as the content of Chol increased above

Figure 2 Insertion of APP into different lipid monolayers

The increase in surface pressure ($\Delta\pi$) after injection of the protein into the subphase was measured as a function of the initial surface pressure π_i . Monolayers of Chol (■), SPM (●), PC (▲) or PS (◆) were prepared.

Figure 3 Effect of Chol on the APP-induced surface pressure increase

Results are expressed as the percentage of surface pressure change of a lipid monolayer after the addition of 4.5 nM APP to the buffer subphase. Monolayers were formed from various ratios of PC, Chol and SPM lipids at $\pi_i = 10$ mN/m. Increasing amounts of Chol were inserted into PC (◆) and SPM monolayers (▲).

50 mol%. If the percentage of increase in $\Delta\pi$ for pure PC is used as a reference (100%), 75 and 100 mol% Chol correspond to increases in $\Delta\pi$ of 197 and 231% respectively. These results indicate that the Chol content influences the insertion of APP into PC monolayers. Similar insertion experiments were performed using SPM monolayers containing a certain molar percentage of Chol (Figure 3). As for the PC–Chol monolayers, the addition of Chol influenced the insertion of APP into the SPM monolayer. The effect is proportional to the Chol concentration in the monolayer. These results indicate that the insertion of APP depends on the ratio of Chol to SPM, as well as on the ratio of Chol to PC.

Protein–lipid monolayers

To evaluate and assess further the stability of the APP–lipid interaction, π – A isotherms of APP-containing monolayers were studied (Figure 4). At any given pressure, the addition of 45 pmol

Figure 4 Surface pressure–area (π – A) isotherms of APP–lipid monolayers

Monolayers were formed from PC (A), Chol (B), SPM (C) or PS (D). Compression isotherms for the pure lipid (---). Compression isotherms with increasing amounts of APP spread on the lipid monolayer: 18 pmol (curve a), 45 pmol (curve b), 90 pmol (curve c) and 135 pmol (curve d).

of APP significantly increased the molecular area of the organized PC monolayer (Figure 4A). By progressively restricting the monolayer area, this effect decreased but did not disappear, showing that APP was not completely expelled from the lipid monolayer. The area was calculated for a lipid molecule, either for pure lipid monolayers or for the protein–lipid monolayers. In the whole range of surface pressures measured, the area per lipid molecule in the presence of the protein was larger than the area per lipid molecule in the absence of the protein (Figure 4). A second compression/expansion cycle led to a very similar isotherm, confirming the stability of the protein–lipid monolayer (results not shown). The isotherms are shifted to an even larger area per molecule with increasing amounts of protein (Figure 4). The amount of APP evoking this effect was relatively low, in the range 18–135 pmol. A proportional effect was observed for all the lipids tested. A comparison between the π – A isotherms of heat-denatured and native APP at the lipid/water interface indicated a smaller molecular area for denatured APP when compared with the native protein (results not shown). This result suggests that specific structural motifs within APP are responsible for insertion in the lipid monolayer. There is evidence that the surface lipid composition affects the physical state and lipid packing at the surface. The shape of the π – A isotherms for PC (Figure 4A) indicate that the PC monolayer is in the LE (liquid-expanded) phase, associated with membrane fluidity, a requisite for the investigation of the protein interaction with lipid membranes. The increase in the molecular area agrees with the insertion of APP into the LE state of PC. In contrast, Chol appears to be in a condensed state in the monolayer film at the air/liquid interface. The molecular area obtained from APP–Chol monolayer was slightly lower than that obtained from APP–PC film (Figures 4A and 4B). At low pressure, the isotherms for SPM monolayers were present in the LE phase, and, after compression, underwent a transition to the liquid-condensed state, since a small kink could be observed at $\pi \approx 14$ mN/m (Figure 4C). In the presence of APP, a slight film expansion could be detected at any surface pressure for SPM, indicating a slight interaction of the protein with the lipid film. APP did not change the shape of the SPM isotherms and was poorly inserted into condensed SPM films. The insertion of APP into a PS monolayer produced a small increase in the apparent molecular area (Figure 4D). This increase is smaller than the observed effect in a PC monomolecular film. To confirm the effect of Chol on the APP insertion into the monolayer, the extrapolated area of APP as a function of Chol content of the monolayer was measured (Figure 5). When the isotherms were recorded with increasing amounts of Chol in a monolayer of SPM, the observed increase in the molecular area ΔA , induced by the addition of APP, reached a maximum for a Chol content of 100% (Figure 5). An effect of Chol on the ΔA recorded in mixed PC–Chol monolayers was also observed. In this case, the extrapolated molecular area of APP was maximum in a pure PC film and minimum in a pure Chol monolayer. For various PC/Chol ratios, intermediate values were obtained (Figure 5). The calculated molecular areas of APP in the presence of different mixed-lipid monolayers are summarized in Table 1. These areas have been calculated at a given surface pressure, assuming that the amount of APP incorporated into the lipid monolayers corresponded to the total amount of the added protein, neglecting the fraction solubilized in the buffer subphase. This approximation seemed to be acceptable, considering the reversibility of the isotherms and the stability of the measurements. We also assume that the area occupied by a lipid molecule is not modified by the presence of the protein. The greatest difference was observed for the molecular areas obtained for APP in SPM-containing monolayers. Thus, at a surface pressure of 5 mN/m, the APP area was 32.5 and 14.6 nm² for PC

Figure 5 Effect of Chol on the APP-induced molecular area increase

Results are expressed as the percentage of molecular area increase in a lipid monolayer after the addition of 45 pmol of APP. Monolayers were formed from various ratios of PC, Chol and SPM and compression isotherms were recorded. Increasing amounts of Chol were inserted into PC (◆) and SPM monolayers (▲).

Table 1 Effect of lipid on the apparent molecular area of APP

The apparent protein molecular areas were obtained for 45 pmol of APP spread on the indicated preformed lipid films (buffer: 20 mM Tris/HCl, pH 7.4/150 mM NaCl).

Surface pressure (mN/m)	Molecular area (nm ² /APP molecule)					
	PC	Chol	SPM	PS	PC-Chol-SPM	PC-Chol-SPM-PS
0	33.4	29.3	16.2	19.5	23	11.2
5	32.5	27.3	14.6	18.5	20	10.9
10	27.5	23.4	13.3	16	16.5	9.5
20	20.3	17	9.8	12.7	11.3	6.1
30	13.7	10.4	6.5	5.3	5.2	2.5

and SPM films respectively. This area decreased during compression to 13.7 nm² for the PC monolayer and to 6.5 nm² for the SPM monolayer.

APP–mixed-lipid monolayers

To establish the biological relevance of our study, and since the effect of Chol on membrane properties is known to be complex, we studied the effect of Chol content in more complex monolayers consisting of PC/Chol/SPM (molar ratio 29:15:6) and PC/Chol/SPM/PS (molar ratio 51:30:12:7). This approach provides an appropriate alternative to plasma membrane with the presence of major membrane components, including phospholipids, sphingolipids and Chol. The molecular area measured for APP insertion into the monolayer of PC/Chol/SPM is larger than the calculated value obtained for the PC/Chol/SPM/PS monolayer (Table 1), which suggests that the presence of negatively charged phospholipids such as PS in lipid films prevents the insertion of APP into lipid monolayers even in the presence of high amounts of Chol.

Effect of subphase ionic strength and pH values on APP monolayer characteristics

The role of electrostatic and/or hydrophobic forces on π – A isotherms was investigated (Figure 6). The increase in ionic strength,

Figure 6 Influence of ionic strength on surface pressure–area (π – A) isotherms of APP–mixed-lipid monolayers

Effect of NaCl concentration in the subphase buffer on the molecular area of APP on lipid monolayers formed from PC (**A**), Chol (**B**), SPM (**C**) and PS (**D**). Lipid on 20 mM Tris/HCl (pH 7.4) with 150 mM NaCl (---), APP–lipid on 20 mM Tris/HCl (pH 7.4) with 5 mM NaCl (---), APP–lipid on 20 mM Tris/HCl (pH 7.4) with 150 mM NaCl (—) and APP–lipid on 20 mM Tris/HCl (pH 7.4) with 300 mM NaCl (— · — · —).

Table 2 Effect of ApoE4 on the APP–lipid insertion

Increase in surface pressure $\Delta\pi$ was measured after the injection of APP, ApoE4 or the APP–ApoE4 complex (1:1 molar ratio) underneath a PC, Chol or SPM monolayer at the initial surface pressure $\pi_i = 10$ mN/m.

	Increase in surface pressure $\Delta\pi$ (mN/m)		
	PC	Chol	SPM
APP	14	31.5	10.5
APP–ApoE4	8.8	24	2.6
ApoE4	6	13	2

from 5 to 150 mM NaCl, did not change the global shape of the isotherms, but increased the distribution of APP between the bulk buffer and the interface at larger apparent phospholipid molecular areas. By further increasing the NaCl concentration up to 300 mM, this effect was not more pronounced. In addition, the molecular area observed in the presence of SPM monolayers was less sensitive to the NaCl concentration (Figure 6C), reflecting the fact that the hydrophobic interactions between APP and SPM are less important compared with those observed for mixed APP–PC (Figure 6A) or APP–Chol (Figure 6B) monolayers. The ability of APP to form stable monolayers with lipids at different electrical states has been checked by spreading the protein on lipid films in aqueous subphases prepared at different pH values (pH 4–7). The recorded π – A isotherms after compression showed no variations in the apparent molecular areas (results not shown).

APP–ApoE4 interaction

The insertion of APP into lipid monolayers was investigated in the presence of ApoE4 to study the possibility that the association of APP with ApoE may depend on direct lipid–protein interactions. As shown in Table 2, the addition of either APP or ApoE4 under a lipid monolayer of $\pi_i = 10$ mN/m resulted in a significant surface pressure increase. However, the values of $\Delta\pi$, produced by inserting ApoE into the monolayer of PC, Chol or SPM, were lower compared with those measured for the insertion of APP into the same preformed monomolecular films. Injection of APP–ApoE complex underneath the preformed lipid monolayer caused a decrease in the measured $\Delta\pi$, compared with the value observed for APP, and the final value obtained did not correspond to either the sum of $\Delta\pi$ for each protein or the value of individual $\Delta\pi$. π – A isotherms were measured for films of Chol spread on buffer before and after the addition of APP–ApoE in a 1:1 molar ratio (Figure 7). The π – A isotherms of the mixed monolayer, obtained with APP–ApoE4 complex on a Chol film, show that the molecular area for mixed APP–ApoE4 is lower than the one observed for insertion of pure APP into Chol monolayer (Figure 7). Again, the presence of ApoE attenuated the ability of APP to interact with the lipid monolayer. It is worth mentioning that the interface is not saturated by the amount of APP used in these measurements (see Figure 4) or with the amount of ApoE (results not shown). The effect of ApoE on the insertion of APP into the Chol monolayer was studied in the presence of 300 mM NaCl in the buffer subphase. The increase in ionic strength led to an increase in the apparent molecular area. High ionic strength could destabilize the ApoE–APP complex and, thus, the π – A isotherm shifted to values near those of the APP–Chol monolayer.

DISCUSSION

The present study analyses the interaction of the integral type I membrane protein APP with lipids. APP is a transmembrane

Figure 7 Surface pressure–area (π – A) isotherms of APP–ApoE–lipid monolayers

Measurement of surface pressure versus area per lipid molecule for monolayers of Chol (---), ApoE–Chol (.....), APP–Chol (—), APP–ApoE–Chol spread on buffer subphase of 20 mM Tris/HCl with 150 mM NaCl (---) and APP–ApoE–Chol spread on buffer subphase of 20 mM Tris/HCl and 300 mM NaCl (— · —).

protein that has recently been shown to display surface activity [24]. The presence of lipid monolayers at the surface enhanced the attraction of APP to the interface, since such monolayers were immediately modified in the presence of 4.5 nM APP, as shown by the immediate $\Delta\pi$ increase. Our previous study showed that the air/water interface was not saturated by this specific protein concentration. Increase in surface pressure reflects the insertion of a part of the protein between the acyl chains of the phospholipids and is not merely due to a peripheral attachment to the lipid headgroups [25].

The compression isotherms of spread APP–lipid monolayers show changes in surface pressure in the large apparent lipidic areas. This increase arose directly from the area occupied by the APP molecules inserted into the lipid monolayers. These results reflect the affinity of the protein for weakly organized lipid films. However, the fact that mixed lipid–protein monolayers can be compressed to a surface pressure above 40 mN/m without significant loss of protein from the monolayer indicates a strong interaction between the protein and the lipid, as reported for other proteins [26,27]. The measured penetration area of APP is too small to assume a deep insertion of the entire hydrophobic surface of the molecule into the membrane surface. It suggests an insertion of a small segment of the protein, which could be orientated more or less parallel to the long axis of the lipids. The linear relation between the increase in area and the amount of protein added is an additional argument for the interfacial localization of the protein. A control experiment with heat-denatured APP indicated a smaller molecular area compared with the value obtained for the native protein. This decrease in area indicates a structural modification specific to the denaturation process. We propose that the conformational change could affect hydrophobic regions of APP, allowing the interaction of denatured APP with the monolayer to become less specific and, as a result, decreasing the insertion of APP into the lipid film. Thus this result suggests that the ability of APP to insert into a lipid monolayer depends on its three-dimensional structure. Analysis of the π – A isotherms of mixed protein–lipid films, which are related to the stability of the protein in the lipid films, showed that APP in-

sertion is modified by an increase in the ionic strength. Increasing the ionic strength has the same effect as increase in the protein hydrophobicity, which facilitated the insertion of APP in the lipid film. The extent of the monolayer expansion depends also on the electric charge borne by the polar head of the phospholipids. Addition of APP to a film of PS induced a weak film expansion, which was still dependent on the surface pressure. Film expansions or $\Delta\pi$ increases were larger for neutral phospholipid monolayers such as PC when compared with PS, indicating that, in addition to hydrophobic forces, electrostatic forces are also involved. For SPM-mixed monolayers, low film expansions were observed. This suggests that electrostatic or hydrophobic forces are involved in the interaction of APP with lipids. The structure of both PC and SPM includes the same hydrophilic phosphoryl choline head group, which is zwitterionic at neutral pH; however, they are chemically different. Therefore the physical state and the nature of the lipid polar head groups also play a role. Addition of APP to the aqueous phase underneath a monolayer of Chol induced an even higher increase in the surface pressure, and the effect was found to be gradually decreased as the initial pressure of the monolayer increased. From a physical point of view, the differential increase in surface pressure represents differential insertion between APP and lipids, and does not simply occur as a result of different levels of accessibility of APP to the surface. The influence of the initial surface pressure on the compressibility of the lipid monolayer demonstrates the high specificity of the interaction as established previously for several other lipids and proteins [26–29]. APP and lipids either formed ideal (random) solutions or were essentially mutually insoluble and occupied separate regions in a heterogeneous monolayer. Additional microscopic observations of the monolayer structure will be necessary to assess whether complete immiscibility or ideal mixing occurs in APP–lipid monolayers.

Experiments with mixed monolayers confirmed that the penetrating power of APP is significantly dependent on the nature of the lipid. The effect, observed using pmol amounts of APP, was dependent on the content of Chol in the phospholipid or in the sphingolipid mixture. Increasing Chol content of lipid bilayers has effects on the physical properties of cell membranes such as increasing ordering and rigidity and decreasing permeability and lateral diffusion [30]. This influence of the rigidity of the lipid molecule results in a lower apparent molecular area in the film of Chol compared with the expanded one of PC, which is probably due to a different orientation of the protein in the various lipid monolayers. Alterations in membrane lipid and Chol content have been reported to modulate the activities of intrinsic membrane enzymes [31–33]. It was suggested that decreased cleavage by the α -secretases could be due to decreased lateral mobility of both enzymes and APP in the membrane, which may affect the contact between the APP and its secretases [34,35]. It is therefore possible that alterations in Chol content in subcellular membranes could promote the uptake of APP in the cell membrane and the efficiency with which APP is trafficked along the secretory pathway. It was recently reported that the membrane insertion ability of A β is critically controlled by the ratio of Chol to phospholipids [36]. As the ratio of Chol to phospholipids increases above 30 mol %, A β can insert spontaneously into lipid bilayer by its C-terminus. In this case, membrane insertion can decrease fibril formation [36]. The Chol to phospholipid ratio of the plasma membrane was in the range 0.5–1, whereas Golgi, endosomal or lysosomal membranes have a lower Chol level. However, this variation in the Chol content cannot solely explain the changes observed in fibril formation of the A β . Biological membranes that contain appreciable amounts of Chol invariably contain high amounts of SPM. The strong sphingolipid–Chol interactions form the basis

for rafts in the plasma membrane. The dependence of toxicity of A β on the association with specific lipid compartments suggested that vesicular composition might be a factor in these processes [6]. The striking insertion ability of APP and its strong lipid specificity are expected to have important implications for the biological activity and metabolism of APP and, therefore, for the induction of A β production and the associated neurotoxicity.

The AD link between ApoE and APP also suggests a functional connection between lipid binding and ApoE or APP function. This possibility needs to be further studied. Formation of ApoE–A β complexes was demonstrated by several studies [14,17,18]. In contrast with previous studies employing A β peptides, we investigated the binding of APP to lipid by $\Delta\pi$ measurements and π –A isotherms in the absence or presence of ApoE. Our study demonstrates that ApoE4 affects the insertion of APP into a lipid monomolecular film, at a constant area, as a result of its association with APP. This shift of molecular area to lower values is due to the presence of ApoE, which binds strongly enough to APP even in the presence of lipid. Binding between the two proteins is dependent on hydrophobic and/or electrostatic forces since this interaction is affected by the presence of high ionic strength. These results led us to suggest the possibility that ApoE4 may partially bind to APP through a region that might overlap the site of APP–lipid interaction. This finding is in good agreement with the results of Haas et al. [16], who reported that ApoE directly binds to APP through the A β hydrophobic moiety. The physiological relevance of the APP–ApoE interaction is still unknown. ApoE–lipoprotein complexes are taken up by both astrocytes and neurons as a means of delivering Chol to cells [37,38]. This uptake is mediated by the low-density lipoprotein receptor-related protein, which also mediated uptake and degradation of the APP secreted from the extracellular space [39]. It is possible that decreased brain levels of sAPP or A β may result from increased clearance triggered by an increased brain ApoE level and/or increased binding of APP to Chol-rich membranes. Finally, given the fact that the region of the APP molecule that appears to mediate ApoE binding overlaps the site of action of α -secretase [15], the intriguing possibility is raised that bound ApoE exerts a protective effect on the APP molecule, which switches the proteolytic metabolism of APP towards the amyloidogenic pathway. The interesting possibility that ApoE is involved in the modulation of the proteolytic processing of APP awaits more detailed investigation.

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Received 11 May 2004; accepted 15 June 2004

Published as BJ Immediate Publication 15 June 2004, DOI 10.1042/BJ20040777