In this study we used the microdialysis technique to investigate the effect of the nicotinic agonist epibatidine (2.5 μg/kg subcutaneous) on nAChR-mediated dopamine release in the frontal cortex and nucleus accumbens of freely moving rats. We found that epibatidine significantly reduced the release of dopamine in both the nucleus accumbens and in the frontal cortex. The effect on DA release was more pronounced in the nucleuse accumbens compared to the frontal cortex. A single dose of epibatidine also caused a significant increase in DOPAC levels in the frontal cortex while no effect was observed in the nucleus accumbens. In addition, the HVA levels were significantly increased in both brain regions. Pretreatment with the nicotinic antagonist mecamylamine (2 mg/kg) partly inhibited the epibatidine-induced decreased release of dopamine in the nucleus accumbens.The findings suggest an opposite effect of epibatidine compared to nicotine on dopamine release in nucleues accumbens and frontal cortex. The finding suggests that different nAChR subtypes may mediate the effect of nicotine and epibatidine on dopamine release respectively.

Chronic treatment with nicotine is known to upregulate the nACRs in brain.We have earlier observed that the nAChRs differ in their magnitude of up-regulation and that the $\alpha4\beta2$ nAChR is more readily up-regulated than the $\alpha3$ nAChRs subunit (Warpman et al., 1998). Nisell et al. (1996) found that subchronic nicotine treatment enchanced the effect seen by nicotine on dopamine release in the frontal cortex but not in the nucleus accumbens. Marshall et al. (1997) however recently reported no significant effect of nicotine on dopamine release in the frontal cortex of rats subchronically treated with nicotine. In the present study we found that treatment with nicotine (0.45 mg/kg base s.c. twice daily) for 7 days counteracted the decrease in dopamine release seen following a single dose of epibatidine while no effect of subchronic nicotine treatment was oberved in the frontal cortex. The findings suggest changes in nAChR subtype properties following repeated nicotine treatment which may change the epibatidine-induced dopamine release. The studies are expected to provide further insight into the modulatory effect of nicotinic receptor on dopamine release in different brain regions and the particular nAChRs subtypes involved in these specific processes.

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34 The segregation of a lipid domain underlies structural and functional modulation of acetylcholine receptor in reconstituted membranes

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Purified acetylcholine receptor (AcChR) from *Torpedo* has been reconstituted into whole asolectin lipids or into defined multicomponent liposomes made of 50% phosphatidylcholine, 25% cholesterol plus 25% of one of the following phospholipids: phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), phosphatidylglycerol (PG) or phosphatidic acid (PA) (Avanti polar lipids). These phospholipids were all derivatives of egg PC and therefore, have the same fatty acid composition.

The ability of the reconstituted samples to promote cation translocation in response to agonist (carbamylcholine) binding was assessed by using a 'stopped-flow /fluorescence quenching' assay of Tl^+ influx. As expected from previously published data in similar lipid media, the samples reconstituted in vesicles made exclusively from zwitterionic PC/cholesterol mixtures completely lacked ion channel activity. On the contrary, the presence in the vesicles of phospholipids other than PC retains AcChR activity to an extent which varies depending upon the phospholipid. Thus, the largest responses to cholinergic agonists observed correspond to samples containing PA (about one half of the maximal response seen in the samples reconstituted in whole asolectin, used as a reference for full functional reconstitution), followed by those containing PG, the other anionic phospholipid used in these studies. Finally, the samples containing the zwitterionic PE exhibited a very low level of cation channel activity.

We are reporting here that specific alterations of the AcChR secondary structure accompany the reported lipid-induced effects on AcChR function. Fourier-transform infrared (FT-IR) studies of the reconstituted samples show that the amide I band in the infrared spectrum of the protein, when reconstituted in the more active, PA-containing matrix, resembles closely that observed in the fully-functional samples reconstituted in whole asolectin lipids, while it differed considerably from spectra obtained from inactive samples reconstituted in PC/cholesterol mixtures. Analysis of the overlapping secondary structure spectral components in the amide I band contour (Echabe et al., 1997) revealed that the maintenance of AcChR function by phospholipids such as PA, can be correlated with the preservation of a high percentage of α-helical components in the protein structure. On the other hand, the absence of such relevant lipids results in inactive reconstituted samples, in which the AcChR protein exhibits a significant loss

of α-helical structure and an increase in non-ordered structural components *(figure 1)*.

The question remains as to determining: i) what are the molecular events responsible for the effects of phospholipids in modulating AcChR structure and function?; and ii) why is PA the most effective phospholipid in this regard? In the former, the observation that lipid effects on AcChR structure and function occur correlatively and involve precisely lipids such as PA, with a higher affinity for binding to the AcChR protein (Fong and McNamee, 1987), suggests that the interaction of the lipids with the transmembrane portion of the AcChR is what causes that the lipid-bound protein adopts a functionally-competent structure, capable to respond properly to the agonists in the opening of the AcChR ion channel. To test such a hypothesis in terms of the involvement of possible changes in lipid organization and dynamics, we prepared samples of AcChR reconstituted in lipid mixtures containing 50% egg PC, 25% cholesterol and 25% of either perdeuterated DMPA (d-DMPA) or perdeuterated DMPC (d-DMPC). Such dimyristoyl derivatives were chosen for these studies because of their convenient phase transition temperatures. FT-IR spectroscopy and differential scanning calorimetric (DSC) studies on the effects of the protein on phospholipid dynamics within these reconstituted samples show that in the absence of protein, the complex population of membrane lipids remains ideally mixed. On the contrary, the presence of the AcChR protein in the reconstituted vesicles directs the formation of specific lipid domains that become segregated from the bulk lipid matrix *(figure 2)*. Furthermore, additional FT-IR and DSC studies indicate that such lipid domains are likely to be composed of monoanionic PA and cholesterol.

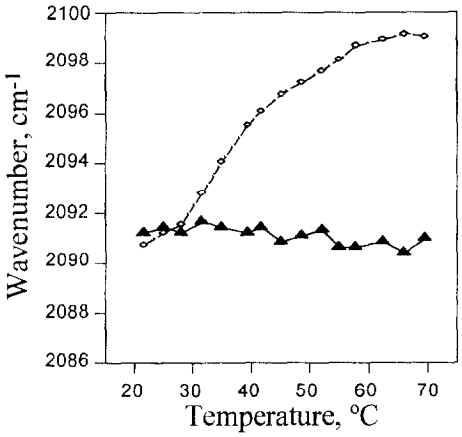

Figure 2. The AcChR protein induces temperature-dependent changes of the CD2 symmetric stretching band of vesicles made from 50% PC, 25% cholesterol and 25% of perdeuterated d-DMPA (diamonds). On the contrary, no temperature-dependent changes can be observed in the absence of protein, suggesting that under conditions, the lipid components are ideally mixed.

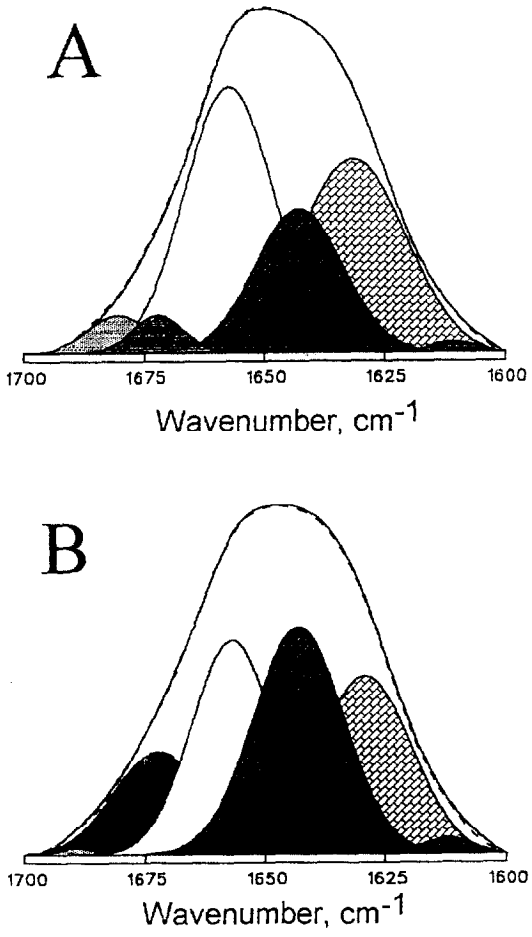

Figure 1. Secondary structure determination from curve-fitting the amide I band of 'functional' (PA + PC + Cho, A) and 'non-functional' (PC + Chol, B) reconstituted samples. Spectral components at 1652 and 1640 cm^{-1} are asigned to α-helical and non-ordered structures, respectively.

As to the selectivity exhibited for PA in this process, it has been reported that it can not be accounted for based on electrostatic interaction with the protein (Raines and Miller, 1993). This, along with the fact that all the phospholipids used in this work have identical acyl moieties, lead to the conclusion that the main contributing factor in the interaction with the lipids should be the recognition of the entire polar head group by the protein. This implies that the near to the lipid-water interphase ends of transmembrane protein segments in contact with the lipid bilayer such as the M4, are likely candidates to be involved in determining the observed selectivity.

As the main conclusion of this report, we propose that a protein-induced, lipid phase separation phenomenon, causing the segregation of specific lipid species, constitutes the basis for the lipid modulation of AcChR structure and function. Partly supported by grant PM95-0108 from the DGICYT of Spain.

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