LIPID-PROTEIN INTERACTIONS IN RECONSTITUTED MEMBRANES CONTAINING NICOTINIC ACETYLCHOLINE RECEPTOR.

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The nicotinic acetylcholine receptor (AcChR) from *Torpedo* is a large transmembrane glycoprotein composed of four different polypeptide subunits (α, β, γ and δ) in a 2:1:1:1 stoichiometry. Binding of cholinergic agonists to extracellular domains on the α subunits, causes the formation of a transient cation channel within the protein, responsible for the initiation of postsynaptic membrane depolarization.

In this report, we have explored the possibility that the well known lipid dependence of AcChR ion channel function is partly due to the occurrence of specific phospholipid-induced modifications of the AcChR protein structure. To this end, purified AcChR protein has been reconstituted in vesicles made from lipid mixtures containing a constant amount of egg phosphatidylcholine (50% by mole) and cholesterol (25% by mole), plus a fixed mole percent (25%) of one of the following phospholipids whose effects on AcChR structure and function were to be tested: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidic acid (PA). These phospholipids were all derivatives of egg yolk PC and therefore, have the same fatty acid composition. Use of such lipid mixtures in reconstituting the AcChR results in the formation of sealed unilamelar reconstituted vesicles, perfectly adequate for rapid ion flux measurements, as well as for Fourier-transform IR spectroscopic studies.

To test the ability of the AcChR to rapidly increase the permeability to cations of the reconstituted membrane in response to binding of cholinergic agonists, we have used a rapid kinetics, "stopped-flow/fluorescence quenching" assay of Tl^+ influx. Our results show that the AcChR reconstituted in vesicles made exclusively from zwitterionic PC/cholesterol mixtures completely lacks the ability to activate the characteristic cation channel in response to cholinergic agonists. On the contrary, the presence of phospholipids other than PC in the reconstituted vesicles, partly retains AcChR activity to an extent which varies depending upon the phospholipid. Thus, the largest responses to cholinergic agonists correspond to samples containing PA (about one half of the maximal response seen in the samples reconstituted in whole asolectin lipids used as a reference for full functional reconstitution), followed by those containing PG, while the samples containing the zwitterionic PE exhibited a very low level of activity.

The FTIR spectra of the above samples compared to that of the protein reconstituted in whole asolectin lipids, shows band-widening and other changes in the spectral shape

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of the amide I band of the AcChR as a consequence of reconstitution in the different lipid mixtures. The observed spectral changes, which must be attributed to alterations in the protein secondary structure, are i) less pronounced in those samples containing PA, ii) most noticeable in samples reconstituted in zwitterionic PC/cholesterol mixtures and iii) of an intermediate magnitude in the PG and PE reconstituted samples. Similar observations can be made in the deconvolved spectra. Again, presence of PA in the reconstituted vesicles determines only minor alteration in the protein deconvolved spectra, while the most functionally perturbed sample, i.e, that obtained in the presence of PC as the only phospholipid component of the reconstituted vesicles, has a clearly distorted deconvolved spectra in which i) the ratio between the absorbance maxima at 1656 cm^{-1} and 1636 cm^{-1} (attributed to α-helical and β-structure spectral components, respectively) is clearly diminished and ii) the shoulder at 1644 cm^{-1}, assigned to non-ordered protein structures, as well as other components appearing in the 1670-1690 cm^{-1} region, become more prominent. Also, as expected from the observations made on the original spectra, the deconvolved spectra of the PE and PG samples exhibit alterations which are somehow intermediate between those described for the PA and PC samples.

In addition to the qualitative information stated above, infrared spectroscopy could also be used to determine quantitatively the protein secondary structure. Smoothing of the spectra using a maximum entropy algorithm, followed by decomposition of the amide I band into its overlapping spectral components by curve fitting techniques (J.L.R. Arrondo, personal communication) shows that the most striking differences between "functional" (asolectin, PA+PC+Chol or PG+PC+Chol) and "non-functional" (PC+Chol or PE+PC+Chol) reconstituted samples is that the latter exhibit a significantly higher proportion of non-ordered structural elements, at the expense of a decreased proportion of α-helical structure. Compared to the spectra of AcChR samples submitted to other experimental conditions also known to produce a reversible loss of the AcChR ion channel activity, such as desensitization by cholinergic agonists or reconstitution in a lipid matrix lacking neutral lipids, the spectral changes reported here affect differently the various spectral components involved, suggesting a certain cause-specificity in the observed alteration of the protein spectral fingerprint.

Perdeuterated phospholipids were also used to study how the organization of specific lipids could be affected by the presence of the protein. To this purpose, the AcChR was reconstituted in vesicles made from 50% egg PC, 25% cholesterol and 25% of either perdeuterated DMPA (d-DMPA) or perdeuterated DMPC (d-DMPC). Plain lipid vesicles of identical composition were also prepared. The frequency of the CD_2 vibration arising from either d-DMPC or d-DMPA in the plain lipid vesicles, as well as that from the samples containing d-DMPC and AcChR protein, did not show any temperature dependance. However, presence of the AcChR protein in the reconstituted vesicles containing d-DMPA causes that the CD_2 vibration becomes temperature-dependent with a major cooperative, temperature-induced event around 35°C, suggesting that the protein induces a drastic change in the way the d-DMPA organizes, which leads to non-ideal lipid mixing within the reconstituted bilayer.

The observations reported here suggest an important and complex role of lipid-protein interactions in the modulation of membrane protein structure and function.