Journal of Molecular Neuroscience

ISSN0895-8696/06/30:121–124/$30.00
JMN (Online)ISSN 1559-1166
DOI 10.1385/JMN/30:1-2:121

ORIGINAL ARTICLE

Structural and Functional Changes Induced in the Nicotinic Acetylcholine Receptor by Membrane Phospholipids

Asia M. Fernández-Carvajal,*[,1] José A. Encinar,[1] José Antonio Poveda,[1] Entilio de Juan,[2] Juan Martínez-Pinna,[2] Isabel Ivorra,[2] José Antonio Ferragut,[1] Andrés Morales,[2] and José Manuel González-Ros[1]

[1]Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, Spain; and [2]División Fisiología, Universidad de Alicante, Alicante, Spain

Introduction

Ligand-gated ion channels (LGICs) constitute an important family of complex membrane proteins acting as receptors for neurotransmitters (Barnard, 1992; Ortells and Lunt, 1995). The nicotinic acetylcholine receptor (nAChR) from *Torpedo* is the most extensively studied member of the LGIC family and consists of a pentameric transmembrane glycoprotein composed of four different polypeptide subunits (α, β, γ, and δ) in a 2:1:1:1 stoichiometry (Galzi and Changeux, 1995; Hucho et al., 1996) that are arranged pseudosymmetrically around a central cation-selective ion channel. Conformational transitions, from the closed (nonconducting), to agonist-induced open (ion-conducting), to desensitized (nonconducting) states, are critical for functioning of the nAChR (Karlin, 2002). The ability of the nAChR to undergo these transitions is profoundly influenced by the lipid composition of the bilayer (Barrantes, 2004).

Despite existing information on lipid dependence of AChR function, no satisfactory explanation has been given on the molecular events by which specific lipids exert such effects on the activity of an integral membrane protein. To date, several hypotheses have been entertained, including (1) indirect effects of lipids through the alteration of properties of the bilayer, such as fluidity (an optimal fluidity hypothesis [Fong and McNamee, 1986]) or membrane curvature and lateral pressure (Cantor, 1997; de Kruijff, 1997), or (2) direct effects through binding of lipids to defined sites on the transmembrane portion of the protein (Jones and McNamee, 1988; Blanton and Wang, 1990; Fernández et al., 1993; Fernández-Ballester et al., 1994), which has led to the postulation of a possible role of certain lipids as peculiar allosteric ligands of the protein.

In this paper we have reconstituted purified AChRs from *Torpedo* into complex multicomponent lipid vesicles in which the phospholipid composition has been systematically altered. Stopped-flow rapid kinetics of cation translocation and Fourier transform–infrared (FT-IR) spectroscopy studies have been used to illustrate the lipid dependence of both AChR function and AChR secondary structure, respectively.

Results and Discussion

Purified AChR from *Torpedo marmorata* has been reconstituted at high (3500:1) phospholipid to protein molar ratio into vesicles containing egg phosphatidylcholine (PC), cholesterol, and different phospholipids (egg PA, egg PG, and egg PE) as probes to explore the effects of phospholipids on protein function and structure by fluorescence and IR spectroscopy.

Agonist-mediated cation translocation through reconstituted AChR was monitored by using a stopped-flow/fluorescence-quenching assay of Tl^+ influx (González-Ros et al., 1984). When AChR is reconstituted in vesicles of PC/cholesterol mixtures or those containing PE, it completely lacks the ability to activate the characteristic cation channel in response

*Author to whom all correspondence and reprint requests should be addressed. E-mail: asia.fernandez@umh.es

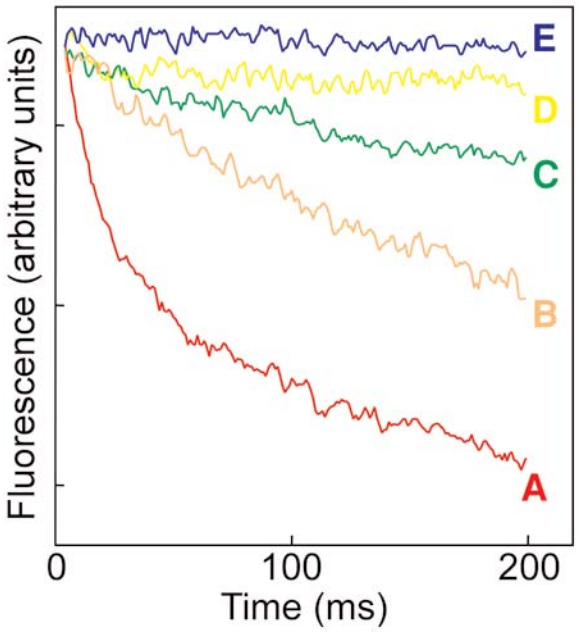

Fig. 1. Stopped-flow recordings of the rapid collisional quenching of 1,3,6,8-pyrenetetrasulfonic acid (PTSA) fluorescence entrapped in reconstituted AChR vesicles by externally added Tl^+. The reconstituted vesicles in 10 m*M* HEPES buffer (pH 7.4), 200 m*M* $NaNO_3$, were rapidly mixed with an equal volume of 10 m*M* HEPES buffer (pH 7.4), 170 m*M* $NaNO_3$, 30 m*M* $TlNO_3$, containing increasing concentrations of carbamylcholine ranging from 0 to 500 µ*M* (final concentration), using a SLM 8000 fluorimeter equipped with a stopped-flow accessory. Shown is a comparison of Tl^+ influx response to 500 µ*M* carbamylcholine in reconstituted AChR membranes prepared from whole asolectin lipids **(A)** or from lipid mixtures containing 25 mol% of cholesterol, 50 mol% of egg PC, and 25 mol% of either PA **(B),** PG **(C),** PE **(D),** or PC **(E)**.

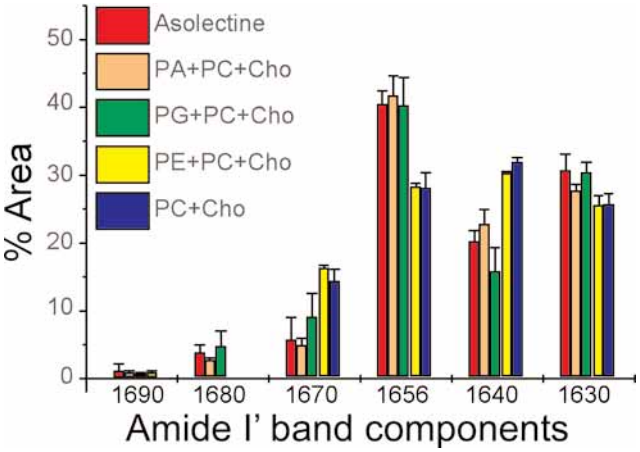

Fig. 2. The diagram shows the statistics of our band-fitting analysis in all the reconstituted samples processed. The main difference between functional (asolectin, PA+PC+Cho [cholesterol], PG+PC+Cho) and nonfunctional (PE+PC+Cho, PC+Cho) reconstituted samples is that the latter exhibit a much higher proportion of nonordered (1640 cm^{-1}) structure at the expense of a decreased proportion of α-helical structure (1656 cm^{-1}). In contrast, the β-structure component at 1630 cm^{-1} seems fairly unaffected by reconstitution in the different lipid media. Also, other changes in minor spectral components, such as the practical disappearance of the band at 1680 cm^{-1} and the increase in the 1670 cm^{-1} band in the nonfunctional samples, can be observed.

to the presence of cholinergic agonists, so these samples are considered nonfunctional. In contrast, the presence of 25 mol% of PA or PG in reconstituted vesicles partly restores AChR activity, reaching—in the case of PA—about one-half of the maximal response seen in the samples reconstituted in whole asolectine lipids and used as a reference for full functional reconstitution. These samples are considered functional (Fig. 1).

These lipid modulations of nAChR function observed in in vitro systems (stopped-flow rapid-kinetics studies) have been corroborated in vivo using the *Xenopus* oocyte model. In these experiments purified nAChRs reconstituted in different lipid matrix are injected into oocytes, where they are efficiently inserted in the plasma membrane. Using the voltage clamp technique we observed that the nAChR in PC/cholesterol reversibly recovered its function and the ACh current was higher when the protein was previously reconstituted in PA. These results suggest that the system is sufficiently dynamic to allow the injected lipid around nAChR to be exchanged for lipids from its own oocyte membrane, but they also suggest that PA must establish a tighter interaction resulting in an increase in protein activity.

To detect possible structural changes associated with lipid nAChR modulation, different spectroscopic studies have been done, mainly through analysis of the FT-IR amide I' band. It is observed that the conformation-sensitive, IR amide I' band in the spectrum of the protein reconstituted in the PA-containing matrix resembles closely that seen in fully functional samples reconstituted in whole asolectin lipids. On the other hand, such spectra differ the most from those obtained from inactive samples reconstituted in egg PC/cholesterol mixtures. Lipid membranes in which the nAChR is fully functional, typically those containing PA and cholesterol, present a higher α-helical content relative to nonordered structure. Conversely, nAcChR reconstituted in lipid membranes in which it is less active, such as those with only zwitterionic phospholipids, the AChR shows a larger proportion

of nonordered structure. Meanwhile, β-sheet content remains basically unchanged in all cases (Fig. 2).

Previous studies from our laboratory have demonstrated an increase in nonordered protein structures under conditions that produce a reversible loss of acChR ion channel activity, such as desensitatization by cholinergic agonist (Castresana et al., 1992) or reconstitution in the absence of neutral lipids (Fernández-Ballester et al.,1994). These observations suggest that a certain disorganization of native protein structures might be a common element in all processes leading to reversible loss of AcChR function. These results also indicate that the preservation of AChR function by specific lipids is accompanied by maintaining specific features in the AChR structure.

Acknowledgments

This work was supported by grant DGI BFI2002-03410 from the Dirección General de Investigación (Spain).

References

Barnard E.A. (1992) Receptor classes and the transmitter-gated ion channels. *Trends Biochem. Sci.* **17,** 368–374.

Barrantes F. J. (2004) Structural basis for lipid modulation of nicotinic acetylcholine receptor function. *Brain Res.* **47,** 71–95.

Blanton M. P. and Wang H. H. (1990) Photoaffinity labeling of the *Torpedo californica* nicotinic acetylcholine receptor with an aryl azide derivative of phosphatidylserine. *Biochemistry* **29,** 1186–1194.

Cantor R. S. (1997) Lateral pressures in cell membranes: a mechanism for modulation of protein function. *J. Phys. Chem.* **101,** 1323–1325.

Castresana J., Fernández -Ballester G., Fernández A. M., Laynez J. L., Arrondo J. L., Ferragut J. A., and González J. M. (1992) Protein structural effects of agonist binding to the nicotinic acetylcholine receptor. *FEBS Lett.* **314,** 171–175.

de Kruijff B. (1997) Biomembranes. Lipids beyond the bilayer. *Nature* **386,** 129,130.

Fernández A. M., Fernández-Ballester G., Ferragut J. A., and González-Ros J. M. (1993) Labeling of the nicotinic acetylcholine receptor by a photoactivatable steroid probe: effects of cholesterol and cholinergic ligands. *Biochim. Biophys. Acta* **1149,** 135–144.

Fernández-Ballester G., Castresana J., Fernández A. M., Arrondo J. L., Ferragut J. A., and González-Ros J. M. (1994) Role of cholesterol as a structural and functional effector of the nicotinic acetylcholine receptor. *Biochem. Soc. Trans.* **22,** 776–780.

Fong T. M. and McNamee M. G. (1986) Correlation between acetylcholine receptor function and structural properties of membranes. *Biochemistry* **25,** 830–840.

Galzi J. L. and Changeux J. P. (1995) Neuronal nicotinic receptors: molecular organization and regulations. *Neuropharmacology* **24,** 563–582.

González-Ros J. M., Ferragut J. A., and Martinez-Carrion M. (1984) Binding of anti-acetylcholine receptor antibodies inhibits the acetylcholine receptor mediated cation flux. *Biochem. Biophys. Res. Commun.* **120,** 368–375.

Hucho F., Tsetlin V. I., and Machold J. (1996) The emerging three-dimensional structure of a receptor. The nicotinic acetylcholine receptor. *Eur. J. Biochem.* **239,** 539–557.

Jones O. T. and McNamee M. G. (1988) Annular and non-annular binding sites for cholesterol associated with the nicotinic acetylcholine receptor. *Biochemistry* **27,** 2364–2374.

Karlin A. (2002) Emerging structure of the nicotinic acetylcholine receptors. *Nat. Rev. Neurosci.* **3,** 102–114.

Ortells M. O. and Lunt G. G. (1995) Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends. Neurosci.* **18,** 121–127.