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LIPID-PROTEIN INTERACTIONS IN MEMBRANES CONTAINING THE ACETYLCHOLINE RECEPTOR

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Lipid-protein interactions involving the acetylcholine receptor (AChR) from the electric ray *Torpedo californica* were studied in purified native membrane vesicles and in reconstituted membranes prepared from receptor protein and defined lipids. The effect of AChR on membrane lipids has been examined by electron paramagnetic resonance (EPR) techniques using spin-labeled lipids incorporated into the membranes. Reconstituted membranes were used to study the dependence of AChR function on membrane lipid composition.

RESULTS AND DISCUSSION

Interaction of Spin-labeled Lipids with Native AChR Membranes

Fig. 1 *a* shows the EPR spectrum of 16-doxylstearic acid in native AChR membranes. The spectrum contains two components. One is due to spin probes that are almost completely immobilized on the conventional EPR time scale. The splitting between the outermost peaks of this component is 62.5 Gauss. The other component is due to relatively mobile spins and is characteristic of 16-doxylstearic acid in fluid phospholipid bilayers (Fig. 1 *d*). The immobile component was quantitated by doubly integrating the spectrum before and after computer subtraction of a computer-simulated spectrum of the component (1). Fig. 1 *b* shows the simulated relatively immobile spectrum and Fig. 1 *c* is the remaining spectrum of the more mobile component after subtraction. Based on the spectral analysis, the amount of immobile probe was 25% and the amount was not strongly temperature dependent over the range 0–20°C. The maximum splitting of the immobile component was temperature dependent and the simulated spectrum was always calculated to match the maximum splitting. The linewidths in Fig. 1 *c* are slightly larger than those of the pure lipid system indicating a small effect of the protein on the bulk lipids.

In contrast to the results with free 16-doxylstearic acid, a phosphatidylcholine spin label (PCSL) containing the 16-doxyl-stearic acid did not clearly show an immobile component when incorporated into native membranes (Fig. 2 *a*). The spectrum is nearly identical to the spectrum of the same probe in lipid-only membrane vesicles (Fig. 2 *b*). Our spin label results are qualitatively similar to those obtained by Rousselet et al. (2) using *Torpedo marmorata*, and indicate that the fatty acid interacts more strongly with AChR than phosphatidylcholine does. We are now examining the spectral properties of phosphatidylserine, phosphatidylethanolamine and cholesterol spin labels to determine if there are charge or lipid-class specificities.

Reconstituted Membranes

Membranes containing purified AChR and defined lipids were prepared by the cholate dialysis procedure developed by Epstein and Racker (3). Using carbamylcholine-stimu-

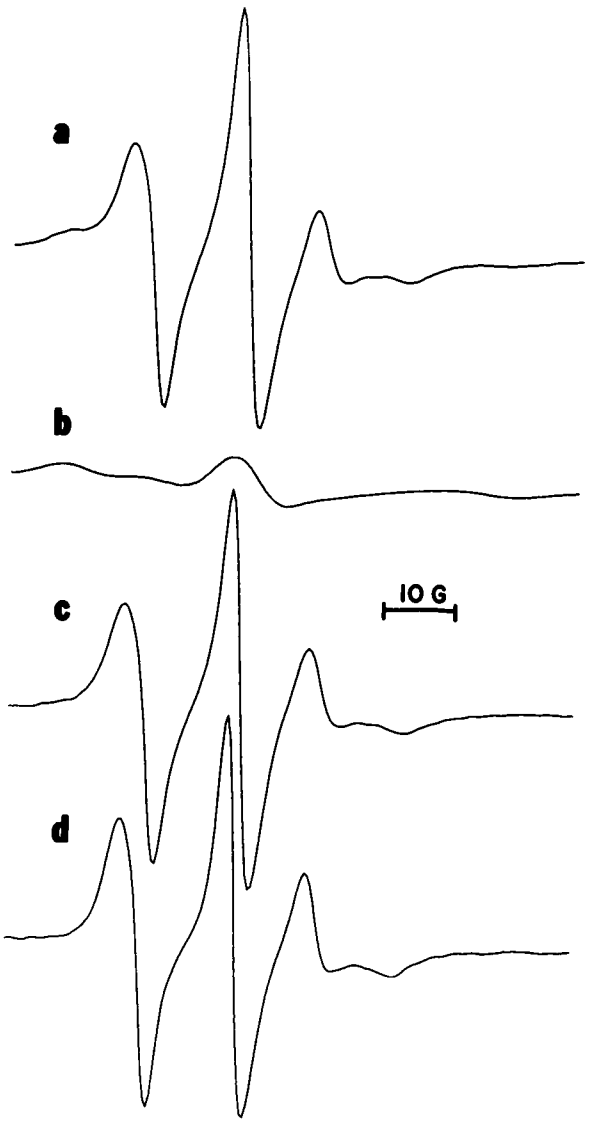

FIGURE 1 (*a*) EPR spectrum of 16-doxylstearic acid in AChR membranes prepared by a method similar to that of Elliott et al. (5) including base extraction. The α-bungarotoxin binding activity was 2,000 pmol/mg protein. The lipid-to-spin probe ratio was 150:1. Incorporation of label was achieved by drying down the spin label in a glass tube and adding membranes. The membranes were pelleted and drawn into a 50 ml capillary tube for spectroscopy. (*b*) EPR spectrum simulated with the Nicolet EPRCAL program. *g* and *a* values were taken from (6). The correlation time was adjusted to give an outer splitting of 62.5 G. (*c*) Spectrum after subtraction. (*d*) Spectrum of 16-doxylstearic acid in liposomes prepared from *Torpedo* membrane lipids.

lated increases in cation flux as the assay for functional receptors, the effect of lipid composition on AChR function was examined. Successful reconstitution required cholesterol in addition to phospholipids (4). The flux activity was proportional to the amount of cholesterol in the range 0–50%. Phosphatidylethanolamine-phosphatidylserine-cholesterol mixtures were found to be best for reconstitution. Phosphatidylcholine alone (with or without cholesterol) did not result in functional vesicles. Asolectin,

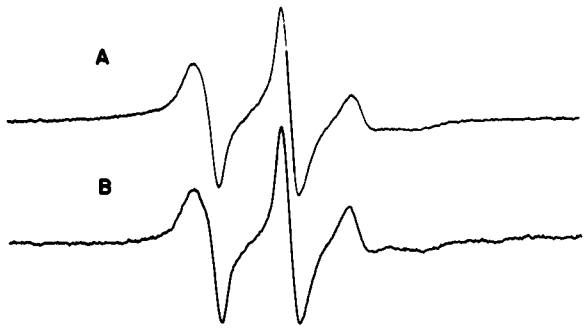

FIGURE 2 (*a*), EPR spectrum of PCSL in AChR. PCSL was prepared according to (7). PCSL was incorporated into membranes by the method of Watts et al. (8). Spectrum was obtained at 0°C. (*b*), EPR spectrum of PCSL in liposomes prepared from *Torpedo* lipids at −3°C.

a crude lipid mixture containing phospholipids and neutral lipids, also gave good results in agreement with many other reported reconstitution experiments.

The reconstituted membranes offer an ideal system for studying lipid-protein interactions. In preliminary studies, 16-doxylstearic acid gave a two component spectrum in reconstituted membranes prepared at a lipid:protein mole ratio of 400:1. The effects of lipids on the properties and relative amounts of the immobilized spin probes are now under investigation.

This work was supported by grant NS13050 from the National Institutes of Health.

Received for publication 4 May 1981.

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