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ORIGINAL ARTICLE

Nicotinic Acetylcholine Receptor Properties are Modulated by Surrounding Lipids

An In Vivo Study

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Introduction

In vitro studies carried out on liposomes of defined composition showed that nicotinic acetylcholine receptors (nAChRs) are fully functional when they are reconstituted in a heterogeneous lipid matrix, such as that provided by crude soybean (asolectin [R-Aso]) lipids. However, when they are reconstituted in plain phosphatidylcholine (R-PC) lipids, their functional activity is completely lost (Fong and McNamee, 1986). This kind of study also pointed out that phosphatidic acid (PA) and cholesterol (Chol) play an important role in preserving the ability of this protein to exhibit an optimal channel activity (Fong and McNamee, 1986). Furthermore, it has been shown recently that nAChR, itself, induces the formation of specific PA-rich lipid domains (Poveda et al., 2002). Because *Xenopus* oocytes incorporate functionally into their plasma membrane nAChRs after intracellular injection of liposomes bearing this protein (Morales et al., 1995), the aim of this work was to determine the effect of the reconstitution lipid matrix on the functional properties of the transplanted nAChRs.

Results and Discussion

Purified *Torpedo marmorata* nAChRs were reconstituted either in whole R-Aso lipids, PC:Chol (75:25 molar ratio; R-PC + Chol), or PA:PC:Chol (25:50:25 molar ratio; R-PA + PC + Chol), and 100-nL aliquots were injected into oocytes. The functional properties of the transplanted nAChRs were assessed by recording the membrane currents elicited by 100 µ*M* ACh (I_{ACh}), under voltage-clamp conditions. Oocyte injection of nAChRs reconstituted in any of these lipid matrices resulted in their functional transplantation into the cell membrane. Nevertheless, the I_{ACh}, recorded at –60 mV, was larger and showed slower desensitization (measured as the percentage of current remaining 2 and 10 s after the current peak) in R-PA + PC + Chol oocytes than in either R-Aso or R-PC + Chol cells (Fig. 1). Besides, a higher apparent affinity of the transplanted nAChR to ACh and a more pronounced voltage dependence of the I_{ACh}, at positive potentials, was found for R-PA+PC+Chol oocytes than those for either R-Aso or R-PC + Chol–injected cells. A change in ion channel selectivity cannot account for the increased I_{ACh} observed in R-PA+PC + Chol cells, as the I_{ACh} reversal potential (approx –5 mV) was not affected by any of the reconstitution lipid matrices.

To determine whether the I_{ACh} increase in R-PA + PC + Chol cells was due to a direct effect of the lipid matrix on nAChR activity or to an increased fusion rate of these proteoliposomes to the oocyte membrane, we preinjected some oocytes with plain liposomes

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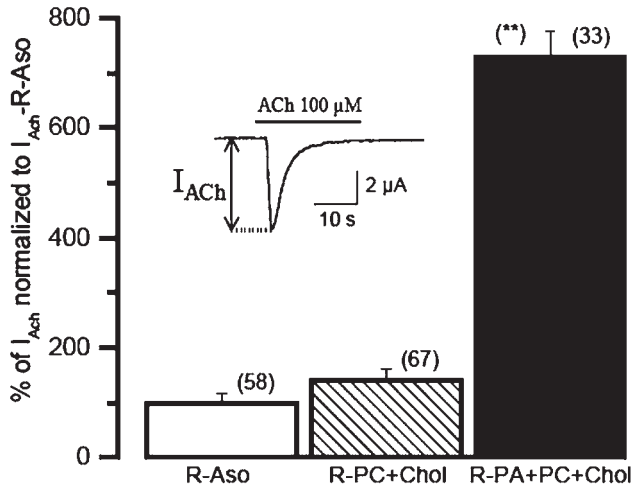

Fig. 1. Bar diagram showing the amplitude of peak ACh (100 µ*M*) currents (I_{ACh}) elicited in ooctyes previously injected with nAChRs reconstituted in R-Aso, a mixture of PC (75%) and Chol (25%; R-PC + Chol) or a mixture of PA (25%), PC (50%), and Chol (25%; R-PA + PC + Chol). Values were normalized to the amplitude of the I_{ACh} obtained in the R-Aso group. The inset shows a representative record of the I_{ACh} recorded in the R-Aso group. The arrow indicates the measurement of I_{Ach}, and the bar, the ACh application time. In all experiments the membrane potential was held at –60 mV. The number of observations is given in parenthesis. Asterisks indicate significant differences with the R-Aso group ($p < 0.01$).

of either Aso or egg-PA lipids (10 mg/mL) 6 h before R-Aso proteoliposome injection. Interestingly, the I_{ACh} of those oocytes that were preinjected with PA liposomes showed a significant increase, but it did not increase in those preinjected with Aso liposomes, confirming a direct effect of PA lipids on nAChR function. Furthermore, the fusion rate of PA lipoproteosomes to the oocyte membrane was found to be similar to that of either R-Aso or R-PC + Chol vesicles, as estimated by the incorporation of ClC-0 channels (a minor contaminant in some of the injected samples).

These results strongly suggest that the transplanted receptors become surrounded by lipids from the oocyte membrane, by exchange, as nAChRs reconstituted in PC+Chol recover their ability to support cation channel activity after transplantation. In addition, this in vivo study confirms previous results from artificial models and supports the fact that nAChRs have a high affinity by PA lipids. This likely results in the formation of a PA-rich domain segregated around the protein, which, in turn, would allow the modulatory effect of this lipid on nAChR function.

Acknowledgments

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