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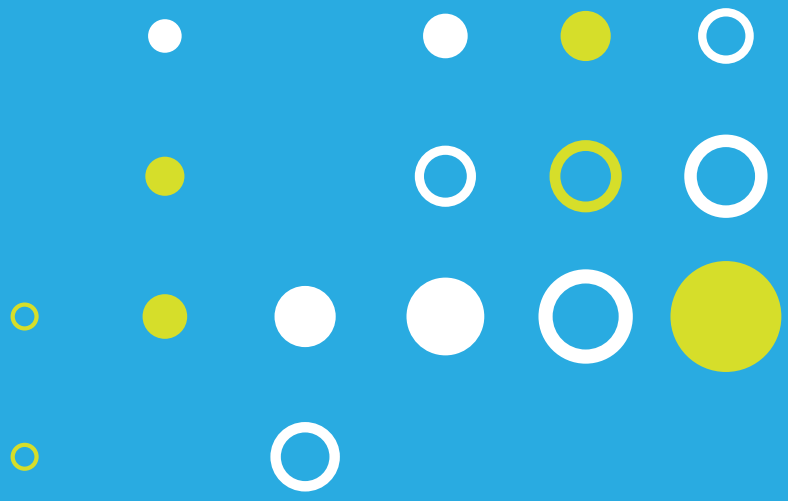

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NS-P05

EFFECTS OF UNSATURATED FATTY ACIDS ON THE CONFORMATIONAL STATE OF NICOTINIC ACETYLCHOLINE RECEPTOR

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Free fatty acids (FFA) are non-competitive antagonists of the nicotinic acetylcholine receptor (AChR) and their site of action is supposedly located at the lipid-AChR interface, where lipids can be annular or non-annular. It is known that the cis-unsaturated FFA, and not trans-unsaturated FFA, produce conformational modifications in the AChR resting state. Using T. californica receptor-rich membranes, we studied the changes in AChR conformational state generated by differences in the double-bond position of monounsaturated FFA. Using the higher affinity of the fluorescent AChR blocker crystal violet for the desensitized than for the resting state, it was observed that a double bond in positions ù 6 or ù 9 increased the KD values of the AChR in the desensitized state whereas no effect was observed in ù 11 or ù 13. Only FFA with an ù 9 double bond changed the KD values in the resting state. DPH and Laurdan fluorescence studies showed that fluidity increased the most in FFA with ù 9 and ù 11 double bonds and that ù 6 and ù 13 had less effect. Fluorescence resonance energy transfer experiments showed that the FFA with an ù 6 double bond remained as an annular lipid whereas all the others also interact at non-annular sites. Thus, the location of the unsaturated double bond appears to be of critical importance for FFA-AChR interaction.

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NS-P06

PHOSPHATIDYLSERINE MODULATION OF AChR LEVELS

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Nicotinic acetylcholine receptors (AChR) are modulated by their lipid environment. The present study was designed to investigate whether AChR function is affected by plasma membrane phosphatydilserine (PS) levels. For this purpose, a mutant PS-deficient cell line, PSA-3, was produced by stable transfection with cDNAs coding for the adult mouse AChR subunits and a plasmid selecting for geneticin. Total RNA was extracted and RT-PCR reactions were performed to verify complete genomic insertion. [125I]á-bungarotoxin radioligand binding assays and fluorescence microscopy studies were employed to study cell-surface and total expression levels and the functional and pharmacological properties of the AChR. Receptor cell-surface expression in the new PS-deficient cell line was stable and depended on PS levels, being reversibly reduced under PS-deficient conditions. Equilibrium and kinetic [125I]á-bungarotoxin binding properties in PS-deficient cells were the same as in control cells. Centrifugation analysis showed a higher proportion of unassembled AChR in PS-deficient cells, which also exhibited higher levels of internalization than normal cells. In conclusion, cell-surface AChR levels are modulated by PS levels.

Supported by grants from MINCyT, CONICET and UNS to FJB.

NS-P07

THE ANTICONVULSIVE DRUG OXCARBAZEPINE IS A NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL BLOCKER

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Oxcarbazepine is an anticonvulsive and mood-stabilizing drug used in the treatment of some forms of epilepsy. Here, we tested the effect of the drug on the ion channel properties of the nicotinic acetylcholine receptor (AChR). Electrophysiological recordings using the single-channel recording patch-clamp technique were used to evaluate AChR function in the presence or absence of different concentrations of oxcarbazepine. The main effects caused by the drug were a concentration-dependent decrease in channel mean open time, an increase in one of the components of the mean burst duration, ôburst, concomitant with a decrease in the duration of the second ôburst component, and the appearance of a new closed-channel component. The duration of the latter remained constant in the range of concentrations tested, although its relative contribution showed concentration-dependent behavior. It is concluded that oxcarbazepine blocks the AChR channel, allowing it to reopen quickly, through a mechanism compatible with that of channel blockers.

Supported by grants from MINCyT, CONICET and UNS to FJB.

NS-P08

DIACYLGLYCERIDES AFFECT DISTRIBUTION AND BINDING PROPERTIES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

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The effects of exogenous and endogenously-generated diacylglycerides (DAG) on the density, affinity, distribution and single-channel properties of the nicotinic acetylcholine receptor (AChR) in CHO-K1/A5 cells were evaluated by a combination of techniques. [125I]-á-bungarotoxin ligand binding assays showed that cells incubated in the presence of palmitoyloleoylglycerol and dioctanoylglycerol (DOG) for 30 min to 3 h augmented cell-surface AChR levels, with a concomitant decrease in the affinity for the?toxin. Longer exposures (18-48 h) decreased AChR density to values between 60 and 80% of those found in control cells. The remaining AChRs accumulated at intracellular compartments. Electrophysiological studies revealed that DOG treatment (30 min) decreased the mean open time of the AChR channel. Increasing the intracellular DAG pool with the diacylglycerol kinase inhibitor D5794, alone or applied together with DOG, promoted a 50 and 100% increase of the cell-surface AChR, respectively. In the latter case, a 50% decrease in the intracellular pool of the receptor was also observed. We conclude that exogenous and endogenous DAGs modulate the expression, distribution and single-channel properties of the muscle-type AChR.

Supported by grants from MINCyT, CONICET and UNS to FJB.