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Regulation of Neuronal Muscarinic Acetylcholine Receptors

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The original goal of this proposal was to determine the effects of electrical depolarization on muscarinic acetylcholine receptors (mAChR) in the cultured neuroblastoma cell line, N1E-115. Previous results in a variety of systems suggested that electrical activity might regulate the number of mAChR in neuronal cells; this research was designed to examine this phenomenon in a defined cell line amenable to growth in cell culture, so that the molecular and cellular mechanisms responsible for these changes could be determined. During the course of these studies, experiments were also undertaken to examine the effects of depolarization on GTP-binding regulatory proteins (G-proteins) and the effects of pertussis toxin, lithium, and phorbol ester treatments on muscarinic receptors in these cells. The gene encoding a neuronal form of the mAChR was isolated, and the function of neuronal mAChR expressed from cloned genes in non-neuronal cells was examined. (AW)

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FINAL REPORT

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ARO Proposal Title: Regulation of Neuronal Muscarinic Acetylcholine Receptors

Name of Institution: University of Washington

Report Prepared By: Neil M. Nathanson

Statement of the Problem Studied

The original goal of this proposal was to determine the effects of electrical depolarization on muscarinic acetylcholine receptors (mAChR) in the cultured neuroblastoma cell line, N1E-115. Previous results in a variety of systems suggested that electrical activity might regulate the number of mAChR in neuronal cells; this research was designed to examine this phenomenon in a defined cell line amenable to growth in cell culture, so that the molecular and cellular mechanisms responsible for these changes could be determined. During the course of these studies, experiments were also undertaken to examine the effects of depolarization on GTP-binding regulatory proteins (G-proteins) and the effects of pertussis toxin, lithium, and phorbol ester treatments on muscarinic receptors in these cells. The gene encoding a neuronal form of the mAChR was isolated, and the function of neuronal mAChR expressed from cloned genes in non-neuronal cells was examined.

Summary of the Most Important Results

Depolarization of N1E-115 murine neuroblastoma cells by incubation with either high potassium or with the sodium channel activator veratridine causes a 50-200% increase in mAChR number. After a 4-8 hour lag, mAChR number increases to reach a new steady state by 20 hours; mAChR number returns to control levels by 24 hours following repolarization. The increase in receptor number occurs in the absence of *de novo* protein synthesis; analyses of the rates of receptor disappearance in cyclohexemide-treated cells in the presence and absence of veratridine indicates that depolarization increases mAChR number due to a decrease in the degradation rate of the receptor. The effects of depolarization can be mimicked by calcium channel blockers and reversed by calcium ionophore, consistent with the hypothesis that membrane depolarization may cause inactivation of voltage-sensitive calcium channels, decreasing calcium levels and/or influx, and thereby altering mAChR metabolism. Depolarization also increases the level of the alpha subunit of the GTP-binding regulatory protein G_o over 24 hours. The level of G_o also returns to control values after 24 hours of repolarization. Neither the alpha subunit of G_i nor the beta subunit of the G-proteins is affected by depolarization. Because of evidence that G_i and G_o may differentially regulate cellular signaling mechanisms, these results suggest that depolarization may regulate specific signal transduction pathways in neuronal cells.

We have found that phorbol esters which activate protein kinase C act synergistically with calcium ionophores to promote the initial internalization and subsequent degradation of the mAChR with the same time course as agonist-induced internalization and subsequent downregulation. Activation of the mAChR also leads to activation of protein kinase C in the cells with the same time course and dependence on agonist concentration as receptor internalization. Long-term treatment with lithium causes increased numbers of mAChR and decreased ability of agonists to induce downregulation. The results are consistent with the hypothesis that the products of phosphoinositide (PI) metabolism and protein kinase C-mediated phosphorylation may regulate

the expression of the mAChR in neuronal cells.

Treatment of N1E-115 cells with pertussis toxin blocks mAChR-mediated inhibition of adenylate cyclase but not mAChR-mediated stimulation of PI turnover. Furthermore, the gene encoding the mouse M1 mAChR was isolated and expressed in Y1 adrenal and L fibroblast cells. The M1 receptor only stimulated PI turnover and did not inhibit adenylate cyclase. In contrast, the M2 receptor when expressed from a porcine cDNA clone only inhibited adenylate cyclase and did not stimulate PI turnover. The M1-mediated response was not blocked by concentrations of pertussis toxin that blocked the M2 response. Thus, different subtypes of mAChR mediate different physiological responses via the action of distinct G-proteins.

Personnel Supported by This Proposal

Craig Brumwell
Jeong Lee
Neil Nathanson
Robert Shapiro

Publications

Liles, W.C., Hunter, D.D., Meier, K.E., and Nathanson, N.M. (1986) Activation of protein kinase C induces rapid internalization and subsequent degradation of muscarinic acetylcholine receptors in neuroblastoma cells. *J. Biol. Chem.* **261**:5307-5313.

Liles, W.C. and Nathanson, N.M. (1987) Regulation of muscarinic acetylcholine receptor in cultured neuronal cells by membrane depolarization. *J. Neurosci.* 7:2556-2563.

Liles, W.C. and Nathanson, N.M. (1988) Altered regulation of neuronal muscarinic acetylcholine receptor induced by chronic lithium treatment. *Brain Res.* **439**:88-94.

Luetje, C.W. and Nathanson, N.M. (1988) Chronic membrane depolarization regulates the level of the guanine nucleotide regulatory protein $G_o\alpha$ in cultured neuronal cells. *J. Neurochem.* **50**:1775-1782.

Martin, J.M., Liles, W.C., and Nathanson, N.M. (1988) Modification of neuronal muscarinic receptor-mediated responses by islet-activating protein. *Brain Res.* **455**:370-376.

Shapiro, R.A., Scherer, N., Habecker, B., Subers, E.M., and Nathanson, N.M. (1988) Isolation, sequence, and functional expression of the mouse M_1 muscarinic acetylcholine receptor gene. *J. Biol. Chem.* **263**:18397-18403.