SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HL 00012-02 LBG

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Muscarinic Acetylcholine Receptors of Cultured Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marshall Nirenberg	Chief, Lab. of Biochemical Genetics	LBG NHLI
OTHER:	William Klein	Postdoctoral Fellow	LBG NHLI
	Orest Hurko	Staff Fellow	LBG NHLI
	Wolfgang Burgermeister	Guest Worker	LC NIAMDD
	Bernhard Witkop	Chief, Laboratory of Chemistry	LC NIAMDD

COOPERATING UNITS (if any)

Laboratory of Chemistry, NIAMDD

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

SUMMARY OF WORK (200 words or less - underline keywords)

Our work on muscarinic acetylcholine receptors focuses on two fundamental questions:

(1) How is binding of acetylcholine to receptors converted into an intracellular response?

(2) What factors regulate receptor concentrations in the membrane?

A binding assay employing tritiated quinuclidinyl benzilate permits us to investigate properties of receptor binding sites and to measure receptor levels in cultured neuroblastoma and hybrid cells under systematically varied conditions.

Project Description:

Objectives: The objectives of our work have been (1) to characterize the nature of ligand interactions with muscarinic receptors, and (2) to assess various factors likely to affect receptor concentrations. Although most methodology involved has been straightforward, our experiments required the custom labeling and purification of muscarinic ligands as commercially available materials were unsuitable due to low specific activity.

Major Findings: The principal findings are:

(1) Excitatory and inhibitory muscarinic receptors for ligands which do not differ pharmacologically.

(2) The affinity of receptors for acetylcholine is affected by the ionic composition of the medium.

(3) Antagonists and agonists differ strikingly in the way that they interact with the receptor. Antagonist binding to receptor follows the law of mass action whereas agonist binding does not. When analyzed on Hill plots, agonist interactions with receptor exhibit negative cooperativity that may be related to receptor desensitization.

(4) The receptor concentration is greatly reduced by exposure of cultured cells to carbamylcholine or other receptor activators. The [receptor·agonist] complex is degraded more rapidly than the free receptor.

(5) Muscarinic excitatory and inhibitory acetylcholine receptors have been solubilized and have been characterized partially.

Significance to Biomedical Research: This work with a model system suggests that, in the normal nervous system, receptor levels, which ultimately influence the pattern of behavior of neural nets, are regulated by synaptic transmission. Some of the events which result from the interaction of acetylcholine with muscarinic acetylcholine receptors have been defined. Two regulatory processes were found which depend upon acetylcholine. One regulates receptor activity, the other regulates receptor concentration.

Proposed Course: Further elucidation of QNB binding will be pursued.