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 00015-01 LBG

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Regulation of cyclic nucleotide biosynthesis by neurotransmitters and opiates

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 Biochem. Genetics	LBG NHLI
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

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

The role of the cyclic nucleotides adenosine 3'5' monophosphate and guanosine 3'5' monophosphate in synaptic transmission is under study using cultured cells of neural origin. The topics of interest during the current year have been the following: 1) The receptor-mediated inhibition of adenylate cyclase activity by alpha-adrenergic agents in neuroblastoma x glioma hybrid cells; 2) Demonstration of a compensatory induction of adenylate cyclase activity in cells treated for one or more days with alpha-adrenergic agents, as had been previously demonstrated by others for opiates, and study of the mechanism of this compensatory induction; 3) The effect of opiates and alpha-adrenergic agents on guanosine 3'5' monophosphate levels in the hybrid cells; 4) Search for a cultured-cell system which synthesizes a peptide with opiate-like properties.

Project Description:

Objectives: Previous work in this laboratory demonstrated that opiates, α-adrenergic agonists, and muscarinic cholinergic agonists lower cyclic AMP (adenosine 3'5' monophosphate) levels by receptor-mediated mechanisms in the neuroblastoma x glioma hybrid cell line NG108-15. Also demonstrated was the inhibition of adenylate cyclase by opiates and a compensatory induction of adenylate cyclase activity (measured in the absence of opiates) in cells grown in the presence of opiates. One aspect of the present project attempts to demonstrate and analyze similar effects of adrenergic agonists on adenylate cyclase in cell-free systems. Another aspect is to relate these findings to the action of the enkephalins, recently discovered endogenous opiate-like peptides.

Major Findings: (1) Adrenergic agents, like opiates, inhibit adenylate cyclase activity in NG108-15 homogenates. The order of agonist potencies is consistent with an alpha receptor, and the effect is blocked by alpha receptor antagonists.

(2) Cultivation of NG108-15 with norepinephrine for 1 to 4 days results, as in the case with opiates, in an increase in adenylate cyclase activity which compensates for the inhibition of this enzyme by norepinephrine. Cells then appear tolerant to norepinephrine, but are dependent upon norepinephrine for maintenance of normal cAMP levels. Withdrawal of norepinephrine or the addition of an alpha-adrenergic antagonist results in an increase in cellular cyclic AMP levels to abnormally high values.

Significance to Biomedical Research: The results show that cells exposed to α-receptor activators for several days become tolerant to and dependent upon these compounds and that tolerance and dependence are established by the mechanism of dual regulation of adenylate cyclase.

Proposed Course: It is intended to continue the investigation of the induction of adenylate cyclase activity by cyclase inhibitors, including the possible mediation of cyclase activators. Also the relationship among the opiate, adrenergic and cholinergic receptors in NG108-15 should be studied. Finally a search for endogenous "endorphin" synthesis in cell lines of neural origin is being initiated, with the goal of developing a tissue culture system for the study of the biosynthesis of this peptide or peptides.