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 00015-02 LBG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of adenylate cyclase by alpha-adrenergic receptors

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

PI:	Marshall Nirenberg	Chief, LBG	LBG NHLBI
OTHER:	Steven L. Sabol	Research Associate	LBG NHLBI
	Saburo Ayukawa	Visiting Associate	LBG NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SE
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The role of the cyclic nucleotides adenosine 3':5' monophosphate (cyclic AMP) and guanosine 3':5' monophosphate (cyclic GMP) 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 alpha-receptor-mediated inhibition of adenylate cyclase activity by norepinephrine in neuroblastoma x glioma hybrid cells; and 2) characterization of a compensatory increase of adenylate cyclase activity in cells treated for 10 hours or longer with norepinephrine and study of the mechanism of this increase, which results in cell tolerance to and dependence upon norepinephrine with respect to cyclic AMP synthesis.

Project Description:

Objectives: Alpha-receptor activators such as norepinephrine rapidly lower cAMP levels of NG108-15 cells by inhibiting adenylate cyclase activity. Furthermore, prolonged exposure of cells to alpha receptor agonists results in an increase in adenylate cyclase activity which compensates for the inhibition. Similar rapid inhibitions and compensatory increases elicited by opiate and muscarinic cholinergic receptor agonists have been recently observed by others. During the past year, attempts have been made to characterize these phenomena further and to elucidate the regulatory mechanisms.

Major Findings: NG108-15 hybrid cells possess α-adrengeric receptors which in concert with receptor activators inhibit adenylate cyclase. Cells were cultured in the presence of norepinephrine for 0-48 hours, then the effects of withdrawal of norepinephrine either by replacing the medium or by the addition of a receptor antagonist was tested. Withdrawal of norepinephrine resulted in a 9-fold increase in cAMP levels of intact cells. Adenylate cyclase activity also increased but to a lesser extent. Studies on the specificity of receptor antagonists showed that both the inhibition of adenylate cyclase by norepinephrine and the subsequent increase in adenylate cyclase activity are mediated by α-receptors. These and other results show that dual regulation of adenylate cyclase is a general phenomenon and that cells can become dependent upon norepinephrine, acetylcholine, or opiates. The cells develop an apparent tolerance to these compounds but in fact remain sensitive to the compound used.

NG108-15 α-receptors were characterized by studying the specific binding of [^{3}H]-dihydroergocryptine and other ligands to the receptors. The specificity of the binding sites for ligands resembles that of α-receptors. The binding of the ligand to the membrane preparation is a saturable process. The average NG108-15 cell possesses 60,000 α-receptors.

Significance to Biomedical Research: 1. The fact that dual regulation of NG108-15 adenylate cyclase has been observed now with three classes of inhibitors each mediated by a different species of receptor, suggests that dual regulation may be a general phenomenon. 2. Norepinephrine released at adrenergic synapses may regulate cAMP levels in post-synaptic or pre-synaptic cells by the mechanism discussed here. Such regulation may modulate the cell's responsiveness to ligands for other species of receptors which activate adenylate cyclase and thus may affect information transfer in the nervous system.

Proposed Course: The potencies of α-receptor activators and antagonists with respect to inhibition of adenylate cyclase will be compared with the effects of ligand binding to NG108-15 alpha-receptors. The mechanism of coupling inhibition of adenylate cyclase with a subsequent compensatory increase in enzyme activity will be studied further.

Effects of α-adrenergic activators and antagonists on [^{3}H] dihydroergocryptine binding will be determined to define the specificity of the α-receptor and the kinetics of binding. The regulation of receptor concentration will be studied.

Publications:

1. Archer, Ellen G., Breakefield, Xandra O. and Sharata, Mary N.: Transport of tyrosine, phenylalanine, trytophan and glycine in neuroblastoma clones. J. Neurochem. 28: 127-135, 1977.