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 00002-04 LBG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Morphine Receptors as Regulators of Adenylate Cyclase

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:	Arthur Lampert	Guest Worker	LBG NHLBI
	James Kenimer	Staff Fellow	LBG NHLBI
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COOPERATING UNITS (if any)

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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 objectives are to elucidate the mechanisms of dependence upon opiates and to define the effects of opiate peptides on cells.

PHS-6040
(Rev. 10-76)

Project Description:

Endorphin peptides were shown to inhibit adenylate cyclase of NG108-15 cells. The inhibition constants (K_i) were 12, 40, 63 and 98 nM for methionine enkephalin, leucine enkephalin, β-endorphin and α-endorphin, respectively. Thus, the endorphins are the most potent peptide inhibitors known and thus, the activations of adenylate cyclase by other species of neurotransmitters or hormones are suppressed. In effect, the opiate peptides act as pleitropic desensitizers of many kinds of receptors, which in concert with the corresponding ligands, activate adenylate cyclase. Exposure of cells to methionine enkephalin for 12 to 97 hours results in an increase in adenylate cyclase activity which compensates for the inhibition of enzyme activity by methionine enkephalin. The cells then have normal cAMP levels and appear tolerant to methionine enkephalin because the increase in adenylate cyclase activity is approximately equal to the inhibition of enzyme activity by the peptide. However, the cells are dependent upon the opiate to maintain normal cyclic AMP levels. Withdrawal of methionine enkephalin removes the enzyme inhibition and reveals abnormally high adenylate cyclase activity. Dual regulation of adenylate cyclase by opiates thus accounts for the phenomena of narcotic dependence and tolerance. Thus, the endorphin peptides and narcotics are pleitropic regulators of cell responses to neurotransmitters and hormones which are coupled to the activation of adenylate cyclase. In this way, opiates can alter the perception of neurons to incoming messages which are destined for adenylate cyclase.

Reactions mediated by the opiate receptors that inhibit adenylate cyclase are closely coupled to subsequent reactions that gradually increase adenylate cyclase activity of neuroblastoma x glioma NG108-15 hybrid cells. Opiate-treated cells have higher basal-, PGE_1-, and 2-chloroadenosine-stimulated activities than control cells. However, NaF or guanosine 5'-(β,α-imido)triphosphate abolish most of the difference in adenylate cyclase activity observed with homogenates from control and opiate-treated cells. Cycloheximide blocked some, but not all, of the opiate-dependent increase in adenylate cyclase activity. These results suggest that the opiate-dependent increase in adenylate cyclase is due to conversion of adenylate cyclase to a form with altered activity. Protein synthesis also is required for part of the opiate effect. A hypothesis is proposed that the activity of adenylate cyclase determines the rate of conversion of the enzyme from a high to a low activity form or via a new opiate, by inhibiting adenylate cyclase.

Highly purified [Leu^5]enkephalin and seven derivatives including [Ala^2, Leu^5]-, [Ser^2,Leu^5]-, [Ser^3,Leu^5]-, [Aba^2,Leu^5]-, and [des-$Gly^{2(3)}$,Leu^5]enkephalin were obtained by solid phase synthesis and their morphine-like activities in neuroblastoma x glioma cell homogenates were measured. Changes at the 2, 3, and 5 positions of the enkephalin provided analogues which were all less active than [Leu^5]enkephalin. The results are discussed in terms of recently suggested conformational structures for the enkephalin peptides. No melanocyte stimulating activity was observed for [Leu^5]enkephalin, [Ala^2,Leu^5]enkephalin, or [Ser^2,Leu^5]enkephalin.

Significance to Biomedical Research. Effects of endogenous opiate peptides on adenylate cyclase activity was defined and molecular mechanisms for the phenomena of narcotic dependence and tolerance were proposed.

Proposed Course: Further studies on the regulation of adenylate cyclase by narcotics and endorphin peptides and the mechanism of coupling inhibition of adenylate cyclase with a subsequent increase in adenylate cyclase activity are in progress.

Publications:

1. Lampert, Arthur, Nirenberg, Marshall and Klee, Werner A.: Tolerance and dependence evoked by an endogenous opiate peptide. Proc. Natl. Acad. Sci. USA 73: 3165-3167.

2. Klee, Werner A., Lampert, Arthur and Nirenberg, Marshall: Dual regulation of adenylate cyclase by endogenous opiate peptides. In: Kosterlitz, H. (Ed.): Opiates and Endogenous Opioid Peptides. Amsterdam, Elsevier/North Holland Biomedical Press, 1976, pp. 153-159.

3. Goldstein, Avram, Cox, Brian M., Klee, Werner A. and Nirenberg, Marshall: Endorphin from pituitary inhibits cyclic AMP formation in homogenates of neuroblastoma x glioma hybrid cells. Nature 265: 362-363, 1977.

4. Agarwal, Nirankar S., Hruby, Victor J., Katz, Robert, Klee, Werner and Nirenberg, Marshall: Synthesis of leucine enkephalin derivatives: Structure-function studies. Biochem. Biophys. Res. Commun. 76: 129-135, 1977.

5. Klee, Werner A. and Nirenberg, Marshall: Mode of action of endogenous opiate peptides. Nature 263: 609-612, 1976.

6. Nirenberg, Marshall: Studies on synapse formation and opiate dependence. J. Natl. Cancer Inst., in press.

7. Sharma, Shail K., Klee, Werner A. and Nirenberg, Marshall: Opiate dependent modulation of adenylate cyclase. Proc. Natl. Acad. Sci. USA, in press.