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 00002-06 LBG

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Receptor Mediated Regulation of Adenylate Cyclase.

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

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COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Section of Molecular Biology

INSTITUTE AND LOCATION
NIH, NHLBI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

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)

Receptor-mediated activation and inhibition of adenylate cyclase of neuroblastoma x glioma hybrid cells and other cell lines were studied.

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Major Findings: The inhibition of adenylate cyclase by morphine and the gradual increase in adenylate cyclase activity that results when NG108-15 cells are incubated for 12 or more hours in the presence of morphine was previously proposed as a model for the analgesic action of opiates and for the phenomena of opiate dependence and tolerance. We now find that linoleic acid or serum lipids are required for the morphine-dependent increase in adenylate cyclase activity, but not for inhibition of the enzyme. Similar results were obtained with norepinephrine which activates α-receptors of NG108-15 cells. In this model system, therefore, the inhibition of NG108-15 adenylate cyclase by morphine or norepinephrine can be dissociated from the acquisition of dependence upon opiates or norepinephrine.

Ten μM morphine or norepinephrine completely inhibit the activation of adenylate cyclase by Ca^{2+} ions, but inhibit basal or PGE_1-activated adenylate cyclase by no more than 55 percent in NG108-15 homogenates. The extent of inhibition of adenylate cyclase by morphine or norepinephrine thus is a function of the Ca^{2+} ion concentration and the proportion of adenylate cyclase molecules that are activated by Ca^{2+} ions.

Activation of serotonin receptors of NG108-15 or NCB-20 hybrid cells by serotonin results in cell depolarization, action potentials, and secretion of acetylcholine into the medium. These responses desensitize in less than 15 sec and are not inhibited or mimicked by LSD. Serotonin also stimulates adenylate cyclase activity of NCB-20 hybrid cells, but this effect of serotonin does not desensitize. Eadie-Scatchard analysis suggests a bimolecular interaction and reveals no evidence of receptor heterogeneity. The Hill interaction coefficient is 1.0, indicating independent, noncooperative reactions. LSD activates adenylate cyclase (K_{act} = 12 nM) and also inhibits the activation of the enzyme by serotonin (K_i - 10 nM). In addition, mianserin and cyproheptadine inhibit serotonin activation of adenylate cyclase (K_i = 43 nM and 95 nM, respectively) and LSD activation of adenylate cyclase (K_i - 100 nM and 64 nM, respectively). These results show that serotonin and LSD interact during activation of adenylate cyclase.

Binding sites for [^{3}H]LSD were detected in NCB-20 homogenates; the K_{Dapp} was 36 nM, the Hill coefficient was 1.0, and the receptor concentration was 385 fmol/mg of protein. [^{3}H]LSD was displaced by serotonin (K_i = 110-180 nM). These results agree well with those found to be mediated by a serotonin receptor responsive to LSD that mediates activation of adenylate cyclase. Two binding sites for [^{3}H]serotonin were detected in NCB-20 homogenates [K_{Dapp} = 200 nM and 3750 nM] and serotonin-LSD interactions also were detected.

We conclude that NCB-20 hybrid cells possess two species of serotonin receptors, one coupled to activation of adenylate cyclase, the other to cell depolarization and acetylcholine release; that activation of adenylate cyclase does not affect the rate of acetylcholine release, and, conversely, that serotonin-dependent cell depolarization does not affect intracellular levels of cAMP or cGMP in the hybrid cells tested.

Significance to Biomedical Research:

The results suggest that fatty acids may be required for cellular acquisition of opiate dependence and tolerance and that the analgesic action of morphine

may be uncoupled from the acquisition of morphine dependence and tolerance.

Publications:

1. McDermot, J., Higashida, H., Wilson, S. P., Matsuzawa, H., Minna, J. and Nirenberg, M. Adenylate Cyclase and Acetylcholine Release Regulated By Separate Serotonin Receptors Of Somatic Cell Hybrids, Proc. Natl. Acad. Sci. USA 76, 1135-1139 (1979).

2. Wilkening, D., and Nirenberg, M. A Lipid Requirement For Acquisition Of Opiate Or Epinephrine Dependence By Neuroblastoma x Glioma Hybrid Cells, J. Neurochem., In Press.

3. Wilkening, D., Sabol, S. L., and Nirenberg, M. Control of Opiate Receptor-Adenylate Cyclase Interactions By Calcium Ions and Guanosine-5'-Triphosphate, Brain Res., In Press.

4. Sabol, S. L., and Nirenberg, M. Regulation of Adenylate Cyclase Of Neuroblastoma x Glioma Hybrid Cells By α-Receptors, I. Inhibition Of Adenylate Cyclase Mediated By α-Receptors, J. Biol. Chem. 254, 1913-1920 (1979).

5. Sabol, S. L., and Nirenberg, M. Regulation Of Adenylate Cyclase Of Neuroblastoma x Glioma Hybrid Cells By α-Adrenergic Receptors. II. Long-lived Increase Of Adenylate Cyclase Activity Mediated By α-Receptors. J. Biol. Chem. 254, 1921-1926 (1979).