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 00009-05 LBG

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cell Recognition and Synapse Formation.

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
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COOPERATING UNITS (if any)

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LAB/BRANCH
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SECTION
Section of Molecular Biology

INSTITUTE AND LOCATION
NIH, NHLBI, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	6	1.5

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)

Regulatory reactions were identified that turn synapses on or off.

1072

PHS-6040
(Rev. 10-76)

Project Description:

Major Findings: The formation of synapses between clonal cells of neural origin, such as NBr10A or NG108-15 hybrid cells, and rat striated muscle cells was found to be regulated. Exposure of hybrid cells for 3-7 days to PGE_1, which results in activation of adenylate cyclase, or exposure to various cyclic nucleotide phosphodiesterase inhibitors, markedly increases the number of synapses formed. The effects of putative neurotransmitters or hormones on intracellular cyclic AMP or cyclic GMP levels, voltage-sensitive Ca^{2+} channel activity, and acetylcholine secretion were determined. Receptor-mediated increases in intracellular cyclic AMP or cyclic GMP levels had no immediate effect on K^+-dependent $^{45}Ca^{2+}$ uptake by cells or on acetylcholine secretion from cells. However, prolonged exposure of hybrid cells to PGE_1 results both in an increase in cellular cyclic AMP and the gradual acquisition by cells of functional voltage-sensitive Ca^{2+} channels. Concomitantly cells acquire the ability to secrete acetylcholine in response to a depolarizing stimulus and can then form functional synapses with muscle cells.

D600 inhibits $^{45}Ca^{2+}$ uptake dependent on 80 mM K^+ (IC_{50} = 2 x 10^{-7} M), but has little or no effect on $^{45}Ca^{2+}$ uptake in the presence of 5 mM K^+. $^{45}Ca^{2+}$ uptake also is inhibited to 10 mM La^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Sr^{2+}, or Ba^{2+}, but not by 10 μM tetrodotoxin, 20 mM tetraethylammonium, or 1 mM 3,4-diaminopyridine.

Other cell lines were found that synthesize acetylcholine but do not form synapses with striated muscle cells. Various types of synapse defects were detected; including defects in voltage-sensitive $^{45}Ca^{2+}$ channels, vesicles, and an additional unidentified reaction that is required for acetylcholine secretion. These results show that cell lines with or without defects in synapse formation can be generated and that voltage-sensitive Ca^{2+} channel activity can be regulated by a receptor-mediated reaction which is coupled to activation of adenylate cyclase, or by inhibition of cyclic nucleotide phosphodiesterase. Voltage-sensitive Ca^{2+} channel activity increases slowly over a period of days and this reaction is required for stimulus-dependent secretion of transmitter and the formation of functional synapses.

To identify molecules required for synaptogenesis or communication across the synapse, hybrid cell lines which synthesize mono-specific antibodies were obtained by fusion of clonal myeloma cells with spleen cells immunized against cells from the nervous system. Some of the hybridoma cell lines that were obtained synthesize mono-specific antibodies of high titre directed against membrane antigens found on some cells from the nervous system that were not detected with cells from other tissues. One of these cell lines, A2B5, synthesizes antibody directed against an antigen that was shown by indirect immunofluorescence to be associated with plasma membranes of most, or all, neuron cell bodies in chick retina; however, the antigen was not detected on axons or dendrites of neurons, on retina Müller cells, or pigment cells, or on cells from non-neural tissues.

Antigen A2B5 activity is relatively stable at 100°C, is insensitive to trypsin, exhibits the solubility properties of a ganglioside, and is destroyed by

neuraminidase. Antibody A2B5 cytotoxicity against retina cells is inhibited by a tetrasialo GQ ganglioside fraction from bovine brain (estimated half-maximal inhibition, 0.2 μM), or N-acetylneuraminic acid (half-maximal inhibition, 5,000 μM), but not by other purified gangliosides tested. These results suggest that the antigen is a GQ ganglioside in plasma membranes of retina neuron cell bodies but not membranes of axons or dendrites.

A solid-phase ^{125}I-Protein A radioassay for anti-cell surface antibodies was devised which employs target cell monolayers cultured on fenestrated polyvinyl chloride 96-well plates ("transfer plates"). The calibrated aperture in the bottom of each well is small enough to retain fluid contents by surface tension during monolayer growth, but also permits fluid to enter the wells when transfer plates are lowered in receptacles containing washing buffer or test sera. To assay for antibodies directed against target cell surface antigens, transfer plates bearing monolayers are inserted into microculture plates with corresponding 96-well geometry, thereby simultaneously sampling 96 wells. This assay allows rapid screening of hundreds of hybrid cell colonies for production of antibodies with desired specificity.

Methyltransferases can be inhibited by S-adenosyl homocysteine or by analogs which either increase S-adenosyl homocysteine levels or inhibit methyltransferases directly such as 3-deazaadenosine(DZA), adenosine-2',3'-deazido-5'-carboxamide (744-99), 5'-deoxy-5'-isobutylthioadenosine(SIBA), and 5'-deoxy-5'-isobutylthio-3-deazaadenosine(DZ-SIBA). In collaboration with P. Chiang and G. Cantoni the effects of these and other compounds on synapses between dissociated chick embryo retina neurons and cultured rat striated muscle cells were investigated to determine whether inhibition of transmethylation affects synapse formation, acetylcholine release, or muscle responses to acetylcholine mediated by nicotinic acetylcholine receptors. The frequency of spontaneous synaptic responses of muscle cells was markedly reduced by these compounds; half-maximal inhibition was obtained with 1.5×10^{-6} M DZ-SIBA, 1.5×10^{-5} M DZA, 3×10^{-5} M SIBA, or 1×10^{-4} M 744-99. DZ-SIBA reduced the frequency of muscle synaptic responses by 50 percent in 3.5 minutes via a reaction which exhibits first-order kinetics. Homocysteine thiolactone, 5-deoxy-adenosine, or tubercidin, which do not increase levels of S-adenosine homocysteine or inhibit methyltransferase activity, do not affect the frequency of spontaneous synaptic responses of muscle cells. However, homocysteine thiolactone potentiates the inhibition of muscle synaptic responses by DZA by 6-fold. These results suggest that a transmethylation reaction may be required for acetylcholine secretion or vesicle cycling in synaptic terminals of neurons.

Significance to Biomedical Research: Cultured cell systems have been established and used as model systems for biochemical and electrophysiological studies on synapses. A reaction was found that regulates synapse plasticity.

Proposed Course: Current studies focus on determining the reactions which are required for synapse formation and termination and factors regulating these reactions.

Publications:

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2. Eisenbarth, G. S., Walsh, F. S., and Nirenberg, M. Monoclonal Antibody To A Plasma Membrane Antigen of Neurons, Proc. Natl. Acad. Sci., In Press.

3. Schneider, M. D., and Eisenbarth, G. S. Transfer Plate Radioassay Using Cell Monolayers To Detect Anti-Cell Surface Antibodies Synthesized By Lymphocyte Hybridomas, J. Immunol. Methods, In Press.

4. Eisenbarth, G. S., Ruffalo, R. R., Walsh, F. S., and Nirenberg, M. Lactose Sensitive Lectin Of Chick Retina And Spinal Cord, Biochem. and Biophys. Res. Comm. 83, 1246-1252 (1978).

5. McGee, R., Smith, C., Christian, C., Mata, M., Nelson, P., and Nirenberg, M. A New Method For Measurement Of The Uptake And Release Of Materials From Cultured Cells. Anal. Biochem, In Press.

6. DeMello, F. G., The Ontogeny Of Dopamine-dependent Increase Of Adenosine 3',5'-cyclic Monophosphate In The Chick Retina, J. Neurochem. 31, 1049-1053 (1978).