

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 00004-03 LBG												
PERIOD COVERED July 1, 1975 to June 30, 1976														
TITLE OF PROJECT (80 characters or less) Studies of Action Potential and Receptor Ionophores														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" data-bbox="138 493 1445 588"> <tr> <td>PI:</td> <td>W. A. Catterall</td> <td>Staff Fellow</td> <td>LBG NHLI</td> </tr> <tr> <td></td> <td>R. Ray</td> <td>MARC Fellow/NIGMS</td> <td>LBG NHLI</td> </tr> <tr> <td></td> <td>L. M. Huang</td> <td>NIH Postdoctoral Fellow</td> <td>LBG NHLI and BP NINCDS</td> </tr> </table>			PI:	W. A. Catterall	Staff Fellow	LBG NHLI		R. Ray	MARC Fellow/NIGMS	LBG NHLI		L. M. Huang	NIH Postdoctoral Fellow	LBG NHLI and BP NINCDS
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COOPERATING UNITS (if any) Laboratory of Biophysics, NINCDS														
LAB/BRANCH Laboratory of Biochemical Genetics														
SECTION Section on Molecular Biology														
INSTITUTE AND LOCATION NHLI, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.7	OTHER: 1												
SUMMARY OF WORK (200 words or less - underline keywords) <p>The objective of this project is to develop biochemical methods for studies of action potential and receptor ionophores leading eventually to isolation of these macromolecules and characterization at both the molecular and cellular levels. Our current efforts are directed toward developing reagents, mainly <u>neurotoxins</u>, which act on the <u>action potential Na⁺ ionophore</u>, preparing radioactively labelled derivatives, and using these reagents to characterize the ionophore at the cellular level and to solubilize and eventually isolate it. The nicotinic <u>acetylcholine receptor</u> ionophore is also under study at the cellular level.</p>														

Project Description:

Objectives: The objectives of this project are (1) to develop biochemical methods for study of action potential and receptor ionophores, (2) to use these methods to study the mechanism of action of these macromolecules at the cellular and membrane levels, and (3) to solubilize, purify, and characterize these ionophores at the molecular level.

Methods Employed: Biochemical assays which measure changes in passive Na^+ influx were used to study the acetylcholine receptor ionophore and the action potential Na^+ ionophore.

Major Findings: Previous results led to the conclusion that (1) the neurotoxic alkaloids veratridine, batrachotoxin, and aconitine activate the action potential Na^+ ionophore by interaction with a single class of sites; (2) scorpion venom activates the ionophore by interaction with a different class of sites; (3) the sites of action of these 2 classes of toxin are allosterically coupled in a highly cooperative manner; and (4) the inhibitors tetrodotoxin and saxitoxin act at a separate site directly involved in ion transport by the ionophore.

The active component of scorpion venom has been purified using its ability to activate the action potential Na^+ ionophore as a specific assay. The toxin is a polypeptide having a molecular weight of 6700, an isoelectric point of 9.8, and lacking methionine and histidine. The purified toxin retains the ability to act cooperatively with each of the 3 alkaloids. It acts reversibly at a single class of sites with an apparent dissociation constant of 1 to 2 nM. The action of the toxin is highly membrane potential dependent. Depolarization of the cells causes a 30 fold increase in apparent dissociation constant. These results suggest that scorpion toxin binds to a voltage sensitive component of the Na^+ ionophore that acts cooperatively in regulating its ion transport activity.

We have prepared an ^{125}I -labelled derivative of scorpion toxin which retains biologic activity. Using this derivative we have detected a small class of saturable binding sites in electrically excitable neuroblastoma cells but not in neuroblastoma cells defective in electrical activity. Binding of scorpion toxin to these sites is voltage dependent as is the effect of the toxin on ion transport activity. Preliminary estimates of the number of sites are in the range of 3 to 6 fmole/mg cell protein or less than 1 site per μm^2 of cell membrane. This labelled toxin derivative appears to provide an important new tool in studies of the Na^+ ionophore.

Significance to Biomedical Research: The results provide new insights into the mechanism of action and regulation of membrane macromolecules involved in information transfer and processing in the nervous system and in maintenance of normal beating in heart.

Proposed Course: Planned investigations include (1) completing the analysis of scorpion toxin binding to excitable membranes of neuroblastoma cells, nerve axons, and heart muscle; (2) preparing labelled derivatives of saxitoxin and comparing binding with scorpion toxin; (3) studying the voltage dependent aspects of scorpion toxin binding in detail and relating them to the electrophysiologic properties of the ionophore; and (4) attempting to solubilize and purify the binding sites for scorpion toxin and saxitoxin and thus isolate the action potential Na^+ ionophore.

Publications:

1. Catterall, W. A.: Activation of the action potential Na^+ ionophore of cultured neuroblastoma cells by veratridine and batrachotoxin. J. Biol. Chem. 250: 4053-4059, 1975.
2. Catterall, W. A.: Cooperative activation of the action potential Na^+ ionophore by neurotoxins. Proc. Natl. Acad. Sci. USA 72: 1782-1786, 1975.
3. Catterall, W. A. and Ray, R.: Interactions of neurotoxins with the action potential Na^+ ionophore. J. Supramolecular Structure, in press.
4. Catterall, W. A.: Purification of a toxic protein from scorpion venom which activates the action potential Na^+ ionophore. J. Biol. Chem., in press.
5. Catterall, W. A., Ray, R. and Morrow, Cynthia S.: Membrane potential dependent binding of scorpion toxin to the action potential Na^+ ionophore. Proc. Natl. Acad. Sci. USA, in press.