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 neurotoxins, which act on the action potential Na⁺ ionophore, 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 acetylcholine receptor ionophore is also under study at the cellular level.
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 a \(^{125}\)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 \(\mu\)m\(^2\) 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:


