Our aim is to study the distribution of nicotinic acetylcholine receptors in intact and cultured tissues of the peripheral and central nervous system in relationship to the development and function of synapses. To this purpose histochemical localization of α-bungarotoxin bound to the receptors is used in conjunction with light and electron microscopy. In the past year we have studied the ultrastructural distribution of receptors on cultured skeletal muscle fibers and have initiated the following investigations: 1) location and characterization of synapses formed by neuroblastoma hybrid cells in culture 2) modification of histochemical methods in order to permit ultrastructural analysis of receptor distribution in the central nervous system and 3) analysis of the distribution of receptors in the visual system of the goldfish with relationship to optic nerve damage and regeneration.
Project Description:

Objectives: Investigators in this laboratory and others have utilized
labelled α-bungarotoxin (αBT) as a label for nicotinic acetylcholine
receptors in intact and cultured skeletal muscle, and in embryonic and mature
retina. The objectives of this study were to devise a histochemical technique
of greater sensitivity and resolution for localizing bound αBT and to apply
this technique to studying the ultrastructural distribution of acetylcholine
receptors in the peripheral and central nervous system during development, in
culture, and in the mature state.

Methods Employed: We have employed indirect immunoperoxidase staining
of cryostat sectioned, teased, or monolayered cultured materials to which αBT
has been bound. These materials are subsequently examined by light and elec-
tron microscopy.

Major Findings: Acetylcholine receptor-rich regions on the surface of
muscle fibers grown in culture had previously been observed by light micro-
scope autoradiography with [125I]-labelled αBT. The appearance of these
regions could be explained either by (1) the localized presence of complex
folds in the plasma membrane or (2) a high local concentration of receptors in
the plasma membrane, unrelated to membrane folding. Using the αBT-immunopero-
oxidase technique with light- and electronmicroscopy we have shown that hy-
pothesis 2 is correct; the plasma membranes of these regions contain at least
7 times the concentration of receptors found in other regions, with no distinc-
tions in cell surface topography.

Significance to Biomedical Research: Knowledge of ultrastructural distri-
bution of acetylcholine receptor is of clear importance in any attempt to
understand the role of neurotransmitters and their receptors in the function
and development of the nervous system. The α-bungarotoxin-immunoperoxidase
technique already has shown promise for the diagnosis and analysis of mecha-
nisms in human neuromuscular disorders.

Proposed Course: (1) We are using the αBT-immunoperoxidase technique
to help locate and characterize the ultrastructure of synapses which have
been detected electrophysiologically in cultures of neuroblastoma hybrid cells
with skeletal muscle fibers. (2) We are developing new reagents to adapt
the histochemical technique to ultrastructural visualization of acetylcholine
receptor sites in mature and developing central nervous system tissues. (3)
We plan to study the distribution of acetylcholine receptors in the visual
system of the goldfish with relationship to the destruction and reformation
of synapses during optic nerve degeneration and regeneration.

Publications:

1. Ringel, S. P., Bender, A. N., Festoff, B. W., Engel, W. K., Vogel, Z. and
Daniels, M. P.: Ultrastructural demonstration and analytical application
of extrajunctional receptors of denervated human and rat skeletal muscle