

## **Ultrastructural Localization of Enteric Nicotinic Acetylcholine Receptors**

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Within the central nervous system, nicotinic acetylcholine receptors (nAChR's) are found on nerve terminals (i.e., presynaptic) as well as on the postsynaptic membrane. Central presynaptic nAChR's function to facilitate or induce neurotransmission. Functional evidence, provided by pharmacological and electrophysiology experiments, have shown that enteric neurotransmission mediated by nAChR's is not abolished by tetrodotoxin, an inhibitor of axonal action potentials, suggesting some nAChR's must be located on enteric nerve terminals. Furthermore, visualization of nAChR's using immunocytochemistry and light microscopy have also suggested that a subpopulation of nAChR's exist on enteric nerve terminals. However, these methods do not directly demonstrate the existence of nAChR's on enteric nerve terminals. Direct observation of enteric nerve terminals can only be achieved by using transmission electron microscopy (TEM). Therefore, the purpose of this study is to directly demonstrate the location of presynaptic nAChR's at sites of enteric neurotransmission by utilizing the techniques of immunocytochemistry TEM.

Healthy male guinea pigs were anesthetized using halothane, and sacrificed by exsanguination and transection of the cervical spinal cord. Proximal and distal colon were excised and immediately placed in oxygenated Krebs' solution. Whole-mount preparations of longitudinal muscle myenteric plexus were grossly dissected and pinned out on Sylgard flats placed in 1.25% glutaraldehyde, 2% paraformaldehyde, and 0.1M phosphate buffer and allowed to fix for at least 12 hours. Subsequently, sections were rinsed three times at ten minutes each in 0.1M phosphate buffer. Sections were then dehydrated through a series of graded ethanol solutions. The sections were infiltrated with epon media overnight, and subsequently embedded in epoxy resin for a 72-hour polymerization. Ultrathin sections (100 nm) were made using trimmed polymerized blocks and a Reichert-Jung ultra microtome and a Delaware diamond knife. Ultrathin sections were placed on Butvar-coated copper grids to reveal general morphology and areas of interest. Subsequent ultrathin sections were placed on Butvar-coated nickel grids for immunocytochemistry. Primary monoclonal antibody (mAb270, Covance) will be used for localization of nAChR's.

Initial results demonstrate that this technique is useful for the direct ultrastructural visualization of enteric ganglia. Enteric neurons are identified by large, rounded nuclei with a prominent nucleolus. Enteric nerve terminals have characteristics such as numerous presynaptic vesicles and pre- and postsynaptic membrane specialization. Use of immunolabeling for nAChR's is expected to directly demonstrate position of these receptors at sites of enteric neurotransmission, some of which may be directly visualized at presynaptic sites. As further proof that mAb270 localized to presynaptic structures is indeed located at sites of neurotransmission, double labeling will be attempted using mAb270 and an antibody selective for a specialized protein that function in the exocytotic release of neurotransmitters (namely, antibody selective for synaptophysin).