

Development of a simplified method for isolation of highly purified enteric ganglia neurones

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The enteric ganglia, which participates in the regulation of gastrointestinal motility is a large and complex division of the peripheral nervous system (Mihara, 1993; Wood, 1994). To facilitate studies of the myenteric neurones through *in vitro* drug screening assays, we developed a simple method for preparing them in high quantities with high degree of homogeneity.

Segments of the ileum obtained from Sprague-Dawley rat neonates were ligated in both ends and incubated for 30 minutes in trypsin (37°C). Cells extracted were assessed for viability via a series of tests using dye exclusion assay. Cell purification methods included selective cytotoxicity with the drug cytosine arabinoside 7 µM (Blennerhassett and Lourenssen, 2000). The different cell populations were identified with regard to morphology and growth characteristics via immunocytochemistry using antibodies to α -smooth muscle actin and 5HT₃ receptors.

Isolated intestinal cells were <85% viable. In culture the cell population consisted mainly of fibroblasts, intestinal smooth muscle cells, and neurones. When cells were treated with cytosine arabinoside it resulted in complete abolition of accessory cells and proliferation of increasing numbers of myenteric neurones throughout the culture. When characterised by dual-label immunocytochemistry, culture preparations did not stain with the antibody to α -smooth muscle actin, but displayed positive labelling of the cells to selective neuronal antibody to 5HT₃ receptors, presumably on enteric ganglia neurones (Liu et al, 2002).

This study provides evidence that our method permits the culturing of large numbers of highly purified neurones. This is a useful system for exploring mechanisms by which neurotransmitters and neuroactive drugs can regulate the electrophysiological properties of enteric ganglia neurones.

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