Development of choline acetyltransferase activity in motor neuron-enriched primary cultures of chick embryo spinal cord

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Recent evidence, involving tissue-culture techniques, has indicated the existence of a motor neuron trophic factor capable of supporting the survival and neurite outgrowth of spinal cord motor neurons (for review see Slack & Pockett, 1983). One difficulty with this approach is the potential of non-neuronal components, within spinal cord preparations, in influencing directly or indirectly motor neuron survival and neurite outgrowth. Schnaar & Schaffner (1981) described a system whereby a cholinergic-enriched population of neuronal cells was prepared from 7-day-old chick embryo spinal cord. Furthermore, survival of this cholinergic-enriched population (designated F1 cells) was found to be dependent upon culture on highly adhesive substrata in the presence of medium conditioned by skeletal muscle myotube cultures. Detectable levels of the cholinergic marker system, CAT, were obtained only after co-culture of F1 cells with skeletal muscle myotubes or with the mouse myogenic cell line G8. In an attempt to further characterize the survival and cholinergic development of this F1 cell population in vitro we have cultured these cells on hydrated three-dimensional collagen gels.

Cholinergic-enriched neuronal cells were prepared from 6–7-day-old chick embryo spinal cord essentially as previously described, except that a one-step iso-osmotic metrizamide density gradient, 6.4% (w/v), was used. F1 cells were seeded at a density of 250,000 cells/cm² on to collagen gels which had been established as previously described by Elsdale & Bard (1972). Cell culture media consisted of Dulbecco's modified Eagle's medium supplemented with 10% (v/v) horse serum, 10% (v/v) foetal calf serum and 2% (v/v) CEE. Fig. 1 demonstrates that under these conditions a proportion of the F1 cell population survived and extended neurites throughout the three-dimensional lattice at least up to 5 days in vitro. Both bipolar and multipolar morphologies were observed; cell bodies were large, with granular cytoplasm and distinct nuclei. Indirect immunofluorescence analysis with a neurofilament-specific monoclonal antibody revealed that greater than 99% of neurites contained neurofilaments thus confirming the neuronal identity of the majority of cells in the culture. Levels of CAT were analysed at days 2, 5 and 7 in vitro using the phase separation technique described by Fonnum (1975). Acetylcholine production was controlled using acetylcholinesterase to obtain a zero value for each determination. CAT activity in the cultures increased over 2-fold during the first 5 days in vitro with a subsequent decrease at 7 days in vitro. Both bipolar and multipolar morphologies were observed; cell bodies were large, with granular cytoplasm and distinct nuclei. Indirect immunofluorescence analysis with a neurofilament-specific monoclonal antibody revealed that greater than 99% of neurites contained neurofilaments thus confirming the neuronal identity of the majority of cells in the culture. Levels of CAT were analysed at days 2, 5 and 7 in vitro using the phase separation technique described by Fonnum (1975). Acetylcholine production was controlled using acetylcholinesterase to obtain a zero value for each determination. CAT activity in the cultures increased over 2-fold during the first 5 days in vitro with a subsequent decrease at 7 days in vitro. This increase compares favourably with that obtained in cocultures of F1 cells with chick muscle myotubes after 6 days in vitro (Schnaar & Schaffner, 1981).

Hence the cholinergic enriched neuronal preparation is capable of survival upon hydrated collaged gels in the absence of any obvious trophic input from skeletal muscle. It remains possible that such trophic influences may be readily available in CEE. Since significant levels of CAT were detectable at 5 days in vitro it seems likely that a proportion of the cells present are spinal cord motor neurons. As the cultures obtained are virtually free of non-neuronal cell contamination the cholinergic-enriched neuronal preparation provides an opportunity to investigate the direct effect of putative substances, present in or from motor neuron target tissues, on motor neuron survival, neurite outgrowth and cholinergic development in vitro.

Abbreviations used: CAT, choline acetyltransferase (EC 2.3.1.6); CEE, chick embryo extract.

Fig. 1. Morphology of cholinergic-enriched neuronal cells at 5 days in vitro
F1 cells were cultured upon hydrated collagen gels in the absence of muscle-conditioned medium. Two representative fields are shown in (a) and (b). The collagen lattice can be identified in the background. The arrow in (a) highlights a neuronal process entering the gel. Cells exhibiting bipolar and multipolar morphologies are apparent. Magnification × 250.