THE USE OF AN ELECTRICALLY COOLED CHAMBER FOR STUDYING AXONAL TRANSPORT IN PERIPHERAL NERVES IN VITRO

K.F. Griffiths and W.G. McLean, School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF.

The intra-axonal transport of cellular constituents, fast axonal transport, is important for nerve function. It can be studied in the rabbit vagus nerve by application of radiolabelled amino acid to the nodose ganglion and incubation of the nerve in vitro in physiological medium. The subsequent distribution of radiolabelled protein in the nerve can be measured by liquid scintillation counting (McLean et al 1975).

The technique has been modified for more accurate measurement of rates and amounts of transported proteins over short time periods. This involves maintaining the ganglion at 37°C for two hours to allow labelled protein synthesis to occur while inhibiting transport in the axons by cooling the nerve trunk to 10°C. Axonal transport of radiolabelled proteins which have accumulated proximal to the cold zone during that time then resumes on warming the nerve trunk to 37°C.

The apparatus consists of a two-compartment plastic chamber containing oxygenated medium 199 : the smaller compartment contains the ganglion, the larger compartment the nerve trunk. Tritiated leucine (5µl; 5µCi) is injected sub-epineurally into the nodose ganglion of the cervical vagus nerve from male albino rabbits and the nerve placed in the chamber in an atmosphere of moist 95% O_2/5% CO_2. The ganglion compartment is maintained at 37°C by means of a current-carrying resistor fixed beneath it; the nerve compartment is maintained at 10°C by means of a Peltier battery. This battery consists of an array of junctions of two dissimilar conductors and acts as a heat pump when current passes through it. After protein synthesis has occurred (2h) the current through the Peltier battery is reversed and the battery acts to warm the nerve compartment to 37°C. At the same time the ganglion compartment heater is switched off. Transport in the nerves is allowed to continue for up to 4h. Nerves and ganglia are then removed, cut into 2.5mm pieces and prepared for liquid scintillation counting.

A peak of labelled proteins was found to move in the vagus nerve at a rate of 14.5± .85mm/h. This axonal transport was inhibited by vinblastine at 10^{-4}M, when the drug was added to the medium in the nerve compartment. Because of the accumulation of labelled material during the incubation at 10°C and the subsequent decrease of protein synthesis in the ganglion, the peak of labelled proteins was sharply defined and transport rates could be measured accurately. This represents an improvement on our original method (McLean et al, 1975) and allows easier and more accurate control of temperature than other methods which involve cold-blockade of transport in vitro (Brimijoin 1975; Hanson 1979). The method is useful for measuring the effects of drugs, e.g. neurotoxic agents on rapid axonal transport in peripheral nerves.

Brimijoin, S. (1975) J.Neurobiol. 6, 379-394
Hanson, M. (1979) Neurosci., 4, 413-416
McLean, W.G. et al (1975) J.Neurochem., 25, 695-698

This work is supported by Wellcome Research Trust.