Has the multivesicular body containing the neurotrophin retrograde transport organelle other components?

I.A. Hendry, L. Harvey, M.W. Weible II, S.K. Kaasinen, A.J. Reynolds, Neuroscience, John Curtin School of Medical Research, Canberra, Act, Australia

Nerve growth factor (NGF) is released from target tissues, internalised by sympathetic nerve terminals via receptor mediated endocytosis and targeted for retrograde axonal transport within a multivesicular body representing signalling organelle. We used biotinylated NGF (bNGF) and fluorescently labelled receptors p75 and TrkA to positively identify the signalling organelle and fluorescently labelled antibodies to dopamine beta-hydroxylase (DBH) to label organelles derived from synaptic vesicles. bNGF accumulated on the distal side of a sciatic nerve ligature, indicating that it was retrogradely transported. When injected into the anterior chamber of the eye all labelled molecules were retrogradely transported to the superior cervical ganglion in vesicle like organelles. However, the proportion of each label varied across the organelle suggesting that it contained subpopulations of vesicles consistent with a multivesicular body. Small GTP-ase Rab proteins characterise the stages of the endocytic pathway and may play a role in the internalisation, targeting and retrograde transport of the NGF containing signalling organelle. Using antibodies against Rab 4, 5a, 5b, 7 and 11, we demonstrated the retrogradely transported signalling organelle containing fluorescent NGF also contains retrogradely transported Rab 4, 5a and 5b. Since these Rab proteins are restricted to early endosomes, the multivesicular body containing NGF originating from the nerve terminal has characteristics of an early endosome.

We examined the retrograde axonal transport of two populations of vesicles in the sympathetic nervous system. Those containing NGF, NT-3 or NT-4 were significantly lighter than those containing DBH. The retrograde axonal transport of NGF is not inhibited by bis-tyrphostin, FK506, dibutyryl-cAMP, thapsigargin, cadmium or nickel whereas the transport of DBH is the reverse. Thus, for these proteins, there are significant differences in the mechanism of vesicular endocytosis and/or nerve terminal regulatory processes that regulate the targeting of signalling vesicles for retrograde axonal transport. However, using gold labelled tracers it appears that these proteins are in separate vesicles in a single multivesicular body in the axon. This suggests a common transport organelle for retrograde signalling.