AXONAL TRANSPORT OF [^{3}H]CHOLESTEROL DERIVED FROM [^{3}H]LEUCINE IN THE SCIATIC NERVE

P. L. WOOD and R. J. BOEGMAN
Department of Pharmacology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Received 10 April 1980

1. Introduction

Cholesterol, a major constituent of nerve membranes and myelin, appears to be relatively stable [1]; however, a number of pools with varying turnover rates have been reported [2]. Axonal transport of cholesterol has been demonstrated in the sciatic and optic nerve of the chicken and mouse [3–5]. Since leucine can be converted into lipids in the central nervous system, with myelin cholesterol accounting for ~60% of the total radioactivity in the lipid fraction [6,7], we examined the composition and axonal transport of [^{3}H]sterol derived from [^{3}H]-leucine.

2. Methods

The motoneurones in the spinal cord supplying the sciatic nerve of hamsters were labelled with 50 μCi [^{3}H]leucine (60 Ci/mmol) as in [8]. Axonal transport of labelled material was measured by removing the sciatic nerve at different time intervals and treating either 3 or 5 mm nerve segments with 5% trichloroacetic acid for 24 h to remove free leucine. The lipid fraction was obtained by extracting each trichloroacetic acid-insoluble fraction obtained above with ether:ethanol (4:1, v/v) and measuring the radioactivity in the organic phase (lipid) as well as that of the residue (protein). A transport profile was obtained by plotting radioactivity nerve segment vs distance from the motoneurones.

In some experiments, 20 mM puromycin was injected into the spinal cord 30 min before [^{3}H]-leucine, while in others the ventral roots supplying the sciatic nerve were cut at the level of the L4 dorsal root ganglion.

Lipids were separated by thin-layer chromatography on silica gel G employing benzene:ethyl acetate (19:1, v/v) [9] and were visualized by exposing the plate to iodine vapour. Cholesterol was detected by spraying the same plate with H_2SO_4:glacial acetic acid (1:1) and heating at 100°C for 2 min. Individual lipids were scraped from the plate into scintillation vials and extracted with 1 ml acidified ether (ether: glacial acetic acid, 25:1). After the addition of counting cocktail [10], the samples were counted to <5% error.

3. Results

Conversion of [^{3}H]leucine in the spinal cord into lipids reached maximal levels in <2 h of labelling the motor neurone pool. Acid hydrolysates of the lipid extract revealed that <0.5% of the lipid label was present as ^{3}H-labelled amino acid. Thin-layer chromatographic analysis demonstrated that the major portion (73.8%) of lipid radioactivity comigrated with standard cholesterol (table 1). Trace amounts of label were associated with cholesterol esters; however, significant levels of radioactivity were

Table 1
Conversion of leucine into lipid by hamster spinal cord in vivo 2 h after labelling the cord with 50 μCi [^{3}H]leucine

	Lipid as % of total lipid ± SD (n = 6)		
Tissue	Cholesterol	Phospholipids	Fatty acids
Cord	73.8 ± 3.2	20.8 ± 2.7	5.4 ± 0.6
Nerve	86.2 ± 2.5	12.4 ± 2.2	1.4 ± 1.0

Address correspondence and reprint requests to: R. J. B.

Elsevier/North-Holland Biomedical Press

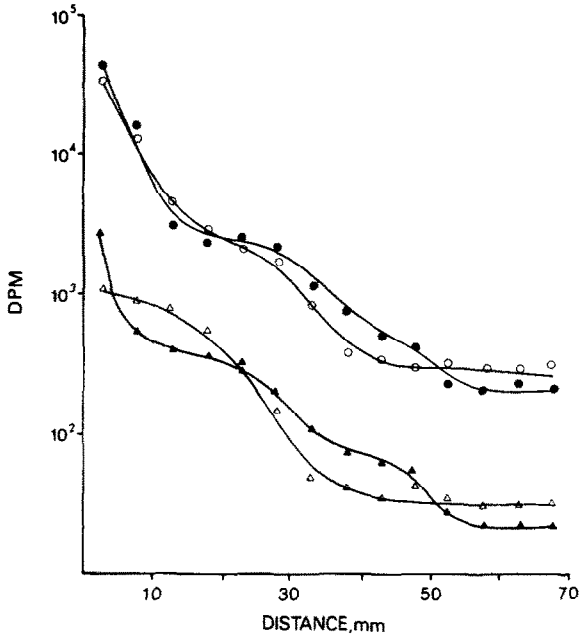

Fig.1. Axonal transport of trichloroacetic acid-insoluble (▲, ●) and lipid (△, ○) radioactivity in hamster sciatic nerve, 2 h (△, ▲) and 3 h (○, ●) after labelling the spinal cord with 2 μl [^{3}H]leucine (50 μCi, 60 Ci/mmol). Results are expressed as dpm/5 mm nerve.

found in the fatty acid (5.4%) and phospholipid fractions (20.8%). These results were confirmed by a second chromatographic solvent system [11] employing light petroleum:ether:acetic acid (70:30:1, by vol.). The lipid present in the sciatic nerve was predominantly cholesterol (86.2%) while only 12.4% and 1.4% comigrated with phospholipid and fatty acids, respectively (table 1).

To determine if ^{3}H-labelled lipid synthesized from [^{3}H]leucine underwent axonal transport, profiles of radioactivity in the sciatic nerve were analyzed at 2 time intervals. Lipid-associated radioactivity at 3 h migrated further down the nerve than that at 2 h as did the trichloroacetic acid-insoluble radioactivity (fig.1), indicating that the lipid underwent rapid axonal transport. The labelled lipid was transported down the nerve in association with protein since the outflow of both protein (trichloroacetic acid precipitate) and lipid radioactivity into the nerve could be prevented by pretreating the spinal cord with 20 mM puromycin (fig.2a). While puromycin caused a 5-fold reduction in incorporation of [^{3}H]leucine into protein (trichloroacetic acid precipitate), it did not reduce the conversion of [^{3}H]leucine into lipids

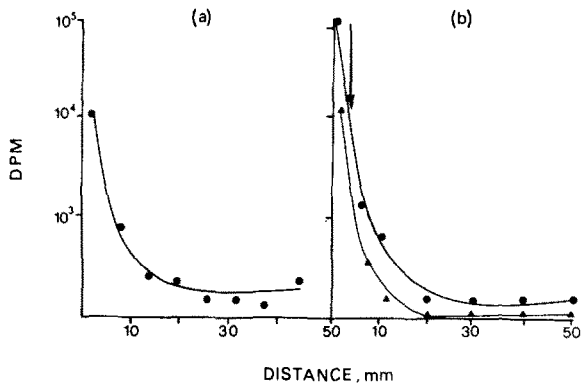

Fig.2. Radioactivity in hamster sciatic nerve 2 h after labelling the spinal cord with 2 μl [^{3}H]leucine (50 μCi). (a) Puromycin (20 mM) was injected into the spinal cord 30 min before [^{3}H]leucine. (b) The ventral roots supplying the sciatic nerve were cut before injecting [^{3}H]leucine. Total trichloroacetic acid-precipitable radioactivity (●) and lipid radioactivity (▲) as dpm/5 mm nerve. The arrow indicates where the ventral roots were cut.

(table 2). On the contrary, in the spinal cord pretreated with puromycin, the conversion of [^{3}H]-leucine into lipid was doubled, presumably due to the increased concentration of free leucine available for metabolism into lipid precursors.

The possibility that the radioactive front observed in our study was due to local cholesterol synthesis by Schwann cells [12] from blood-borne radioactivity was excluded by cutting the ventral spinal roots supplying the sciatic nerve. In these experiments [^{3}H]cholesterol could not be detected in the sciatic nerve (fig.2b). The appearance and axonal transport of lipid radioactivity was not observed when [^{3}H]proline or [^{3}H]valine were injected into the spinal cord, indicating that the radioactivity present in the lipid extract was not due to contamination by ^{3}H-labelled amino acid.

Table 2
Incorporation of [^{3}H]leucine (50 μCi) into protein and lipid by hamster spinal cord in the presence or absence of 20 mM puromycin (N=4)

	dpm ± SD (% of total)		
Puromycin	Protein	Lipid	Trichloroacetic acid-soluble
+	14.8 ± 3.8	12.1 ± 1.4	73.1 ± 2.4
–	80.0 ± 4.3	5.3 ± 1.0	14.7 ± 3.7

4. Discussion

The synthesis of cholesterol from leucine relies upon the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) [13] and the subsequent utilization of HMG-CoA by the isoprenoid pathway. The shuttle of HMG-CoA into the isoprenoid pathway with little degradation to acetoacetate and acetyl CoA is due to the low level of HMG-CoA acetoacetate lyase (EC 4.1.3.4) in nervous tissue [14]. This is supported by the absence of radioactivity in acetoacetate obtained from our lipid extracts of spinal cord or nerve.

The transport of ^{3}H-labelled lipid derived from leucine in the sciatic nerve of the hamster followed a pattern similar to that reported for the transport of [^{14}C]cholesterol in the dystrophic mouse [5]. In our studies on the hamster and those reported for the mouse [5], the lipid was found to migrate with the protein front.

Since nerve ligation or administration of puromycin prevents the appearance of cholesterol in the peripheral nerve, our data indicates that the radioactive cholesterol in the axon was not due to local synthesis from blood-borne [^{3}H]leucine. Local synthesis of cholesterol in the axon from [^{3}H]protein degradation products would lead to a dilution of the transported label, causing a reduction in the transport rate of [^{3}H]cholesterol which was not observed. In addition the transport of cholesterol appeared to be coupled to the transport of protein because inhibition of protein synthesis which leads to cessation of axonal transport [15] also prevented the transport of cholesterol under conditions in which synthesis of sterol was not inhibited. The transport of cholesterol in association with protein is therefore similar to that of phospholipids [16] and glycolipids [17] where export is blocked by inhibitors of protein synthesis. Since the bulk of material undergoing fast axonal transport is in the form of intra-axoplasmic and synaptic plasma membranes [15], the transport of cholesterol synthesized from [^{3}H]leucine would be expected to be in the form of an assembled membrane structure similar to that suggested for phospholipids [16] and glycolipids [17].

Acknowledgement

This research has been supported by the Muscular Dystrophy Association of Canada.

References

[1] Davison, A. N. and Dobbing, J. (1968) Applied Neurochemistry, Blackwell, Oxford.
[2] Kabara, J. J. and Okita, G. T. (1961) J. Neurochem. 7, 298–304.
[3] McGregor, A., Jeffrey, P. L., Klingman, J. D. and Austin, L. (1973) Brain Res. 63, 466–469.
[4] Rostas, J. A. P., McGregor, A., Jeffrey, P. L. and Austin, L. (1975) J. Neurochem. 24, 295–302.
[5] Tang, B. Y., Komiya, Y. and Austin, L. (1974) Exp. Neurol. 43, 13–20.
[6] Ramsey, R. B. (1976) Biochem. J. 158, 501–504.
[7] Smith, M. E. (1974) J. Neurochem, 23, 435–438.
[8] Boegman, R. J., Desphande, S. S. and Albuquerque, E. X. (1980) Brain Res. in press.
[9] Flint, A. P. F. and Denton, R. M. (1969) Biochem. J. 112, 243–254.
[10] Anderson, L. E. and McClure, W. O. (1973) Analyt. Biochem. 51, 173–179.
[11] Kelley, T. F. (1966) J. Chromatog, 22, 456–457.
[12] Eliasson, S. S. and Hughes, H. H. (1969) Neurology 10, 143–147.
[13] Dagley, S. and Nicholson, D. E. (1970) in: An Introduction to Metabolic Pathways, p. 217, Blackwell, Oxford.
[14] Edmond, J. (1974) J. Biol. Chem. 249, 72–80.
[15] Schwartz, J. H. (1979) Ann. Rev. Neurosci. 2, 467–504.
[16] Grafstein, B., Miller, J. A., Ledeen, R. W., Haley, J. and Specht, S. (1975) Exp. Neurol. 46, 261–281.
[17] Sherbany, A. A., Ambron, R. T. and Schwartz, J. H. (1979) Science 203, 78–81.