RETROGRADE AXOPLASMIC TRANSPORT: ITS CONTINUATION AS ANTEROGRADE TRANSPORT

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1. Introduction

Retrograde (cellulipetal) transport of proteins, enzymes and organelles within the axon has been demonstrated under several experimental conditions [1–5]. It is generally accepted that the retrograde transport is inferior to the anterograde (cellulifugal) transport in respect to both the amount of transported materials and transport rate. However, the relation between the two transports in opposing directions is not well understood. The paucity of our knowledge on the retrograde transport is due primarily to a limited applicability of radioisotope, which has been a powerful tool in analysing dynamic aspects of anterograde transport [6].

Bray et al. [7] have shown that the anterograde transport of labelled proteins in the motor fibre of white leghorn pullet is reversed when confronted by an obstruction or on reaching the terminal region of the nerve. We observed similar reversal of polarity in the terminal region of the sensory nerve (dendritic zone) of the frog. The retrograde transport is, like anterograde transport, blocked by colchicine. A minor decrease of a protein component whose mol. wt. approximates 23 000 is consistently observed in the retrograde transport.

2. Materials and methods

L-[4,5-^{3}H]Leucine (4 μCi in 0.2 μl) was injected into the ninth dorsal root ganglion of the bullfrog (*Rana catesbeiana*), and after various treatments shown in fig. 1, radioactivities in 5 mm-long segments of the sciatic nerve were determined as described previously [8]. For polyacrylamide gel electrophoretic analysis of labelled proteins, nerve segments obtained as described in legend of fig. 2 were treated in the following way. Each segment was cut into small pieces and ground in a small all-glas homogeniser in 100 μl of the solution containing 1% sodium dodecylsulphate, 1% β-mercapto-ethanol, 2 mM ethylenediaminetetraacetate and 0.01 M Tris–HCl, pH 7.4. Homogenate was heated in boiling water for 10 min, and insoluble materials were removed by centrifugation. Approx. 90% of the total radioactivity was recovered in the supernatant. A portion of the supernatant was subjected to electrophoresis according to Fairbanks et al. [9], in the gel (0.6 cm × 10 cm) containing 7.0% acrylamide, 0.18% *N,N'*-methylenebisacrylamide and 1% sodium dodecylsulphate. The gels were stained with Coomassie Brilliant Blue R-250, and destained as described [9]. Gel was then cut into 2 mm slices, and each slice was incubated in a vial in 1 ml of Soluene-350 (Packard Instrument Co.) for 4 hr at 60°C. Vials were kept at room temperature overnight before the addition of toluene-based scintillation fluid [8]. Mobility is expressed relative to the tracking dye (Pyronin G) that migrated 9 cm from the origin.

Results and discussion

Thirty hours at 25°C after the isotope injection, rapid anterograde flow of labelled proteins migrating at the rate of 152 mm/day at 25°C [8] had already passed, leaving a low radioactivity (approx. 1000 cpm per 5 mm of nerve) evenly distributed in the nerve portion more than 60 mm distal to the ganglion.

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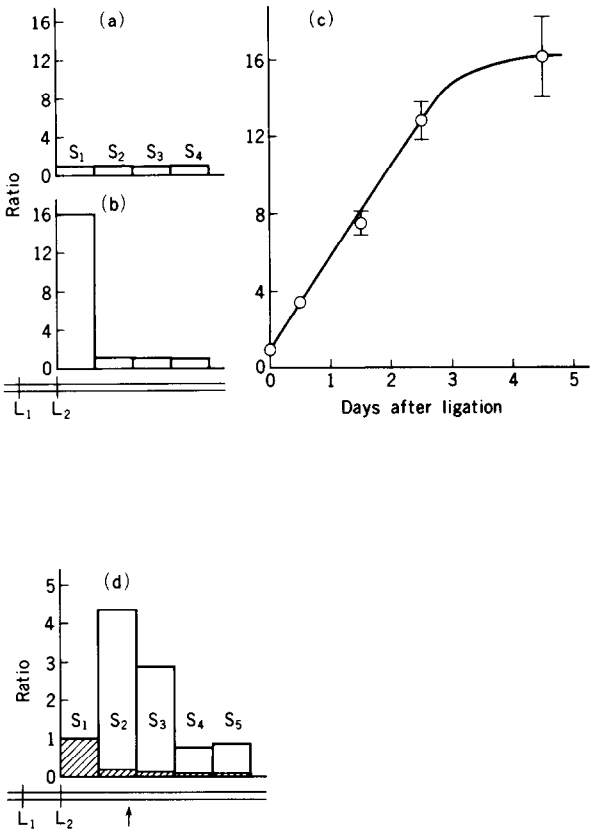

Fig. 1. Accumulation of radioactivity in the nerve portion distal to ligature. Thirty hours at 25°C after the injection of L-[4,5-^{3}H]leucine (4 μCi in 0.2 μl) into the ninth dorsal root ganglion of the frog, the sciatic nerve was ligated approx. 70 mm away from the ganglion (L_1), and after further 6 hr, the second ligature (L_2) was placed 5 mm distal to L_1. Sciatic nerves were removed from animals kept at 25°C for indicated periods, and the radioactivity in each of four consecutive segments (S_1, S_2, S_3 and S_4) was measured. On the vertical axis, radioactivity in each segment is expressed in the ratio, taking the average counts per minute in S_3 and S_4 segments as unity. All values are means of 6–8 experiments. In (a), nerve was removed immediately after placing L_2. In (b), nerve was removed 4.5 days after placing L_2. In (c), the radioactivity ratio in S_1 segment is plotted against the time after L_2. Vertical bars indicate ± S.D. (d) Blockage of the retrograde transport by colchicine. Subsequent to the second ligation (L_2), 5 μl of 0.56 M colchicine dissolved in 0.1 M Tris–HCl (pH 7.5)-ethanol (4:1, v/v) (open columns) or of the solvent only (hatched columns) was injected to the site indicated by an arrow. Animals were killed 3 days after colchicine injection. Radioactivity in each segment is expressed relative to that in respective S_1 segments, which is taken as unity.

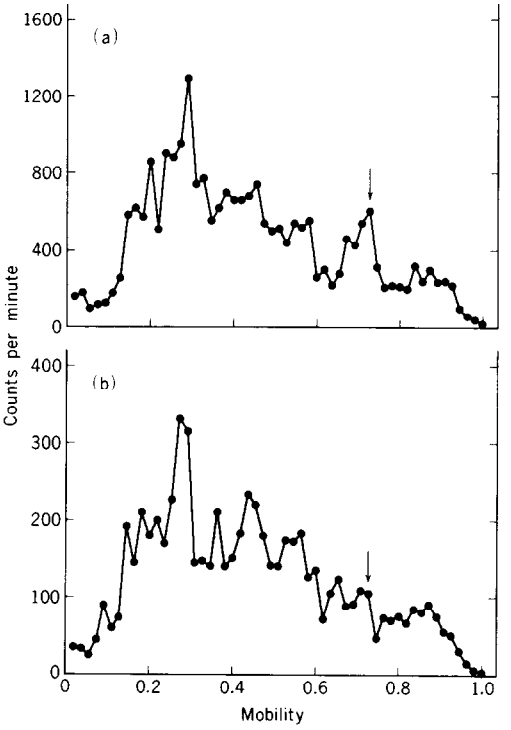

Fig. 2. Polyacrylamide gel electrophoresis of labelled proteins. (a) Anterograde transport. Sciatic nerve was ligated 70 mm away from the ganglion 2 hr after the isotope injection, and after a further 28 hr, 5 mm-long segment just proximal to the ligature was removed and analysed as detailed in the Materials and methods. (b) Retrograde transport. S_1 segment described in fig. 1b (removed 4.5 days after the ligation) was used.

Contribution of slower anterograde transport to this residual activity is unlikely because of the virtual absence of significant protein transport with intermediate flow rates between 152 mm/day and 40 mm/day at 25°C [8].

Thirty hours at 25°C after the isotope injection, the sciatic nerve was ligated with a thin cotton thread at the site approx. 70 mm away from the ganglion (L_1). After a further 6 hr, the second ligation (L_2) was placed 5 mm distal to L_1, and the radioactivity in each of four consecutive 5 mm-long segments (S_1, S_2, S_3 and S_4) distal to L_2 was measured at various time points (figs. 1a and 1b). The first ligature was placed to obviate an incidental contamination of radioactivity to the S_1 segment from the proximal end. To normalise a variation in radioactivity in individual

nerves, radioactivity in each segment is expressed in the ratio, taking the average count in S_3 and S_4 segments (500–1000 cpm) as unity. The ratio in S_1 segment increases linearly with time for three days after the second ligation (fig. 1c), indicating a steady cellulipetal transport of labelled proteins. The increase in the ratio levels off after 4 days, suggesting a rather sharp demarcation of the transport.

The maximal amount of radioactivity accumulated in the S_1 segment via the retrograde transport was approx. 1.5% of the radioactivity found in the ganglion 36 hr after the isotope injection. Since the radioactivity which was transported rapidly in the anterograde direction amounted to approx. 6% of that remained in the ganglion 36 hr after the injection (cf. [8]), it is roughly estimated that one fourth the amount of the anterograde transport shifts over to the retrograde transport.

Colchicine injected in the nerve approx. 10 mm distal to L_2 blocked the retrograde transport, as shown by an unusual accumulation of radioactivity in segments adjacent to the injection (fig. 1d, open columns). In control nerve that received only the solvent, normal accumulation occurred at the distal end of L_2, but no accumulation at the injection site (fig. 1d, hatched columns). Sensitivity of retrograde transport to colchicine has been shown in other experimental systems [10,11]. Vinblastine is also reported to block the retrograde axoplasmic transport examined in vitro [12]. Colchicine has been shown to affect the polymerisation and depolymerisation equilibrium of microtubules both in vivo [13] and in vitro [14,15]. Since it is generally accepted that microtubules are somehow involved in the mechanism of axoplasmic transport [16,17], blockage by colchicine of the transport in either direction implies the occurrence of microtubules with opposing polarities, or a factor that determines the polarity of microtubule action.

Polyacrylamide gel electrophoretic pattern of labelled proteins migrating in the anterograde direction is shown in fig. 2a, which may reasonably be compared with that reported by others [18]. After switching direction, a protein component with mol. wt. of approx. 23 000 was consistently decreased (fig. 2b, indicated by an arrow). The possibility that the decrease is due to a proteolytic degradation during the experimental period appears quite unlikely because the change is selectively confined to this fraction.

In the present experiment, continuity of the anterograde and retrograde transports is demonstrated, but the site at which the reversal occurs is not directly indicated. Reversal en route to the axon terminal is not negated, but is thought to be quantitatively insignificant for the following reasons. First, the maximum amount of radioactivity accumulated in the distal end of the ligature via the retrograde transport remains almost constant irrespective of whether the ligature is placed far from or near the termination of the sciatic nerve. If an appreciable portion of the anterograde transport reverses direction en route to the terminal, the extent of accumulation is expected to be greater in the case of proximal ligation than in distal ligation. Second, in confirmation of the result reported by Bray et al. [7], we have observed that another ligature placed 50 mm distal to L_2 prevents the accumulation of radioactivity in the S_1 segment.

On the basis of the slope shown in fig. 1c, the rate of retrograde transport is calculated to be 24 mm/day, provided that all the radioactivity in respective segments moves upward at a constant rate. However, if a fraction of radioactivity is unmovable, the rate becomes greater than thus calculated. Edström et al. [12] have estimated that the rate of retrograde transport can not be less than half the anterograde rate.

The continuity of the retrograde transport to the anterograde transport may provide a feedback mechanism of informations conveyed from neurone perikaryon to the terminal. Also, it is tempting to speculate that a vehicle for migratory materials runs back toward the cell body, either empty or with molecules that are no longer needed at the terminal. A more satisfactory analysis of transported molecules remains to be made.

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