Local Supply of Energy to the Fast Axoplasmic Transport Mechanism

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ABSTRACT Local anoxia of a small region of cat sciatic nerves causes a block of fast axoplasmic transport in vitro. Activity becomes dammed up at the face of the anoxic region, and falls off rapidly distally just inside the anoxic region. Such blocks were reversible if they were removed within 1 hr. Earlier studies had given evidence that fast axoplasmic transport is closely dependent on ATP derived from oxidative metabolism. The present results indicate that ATP is required all along the length of the nerve fiber to supply a hypothesized "transporting filament" mechanism of fast transport.

A fast transport system present in mammalian nerve fiber carries down protein and particulates from their site of synthesis in the neuron cell bodies (1–4). This was definitively shown in cat sciatic nerves after the dorsal root L7 ganglia were injected with [3H]leucine. A crest of radioactivity was found to move down the nerve at a regular rate of 401 ± 35 mm/day (5, 6).

Fast axoplasmic transport does not require the soma; it is due to a mechanism locally present in the axons (7). It can also take place in vitro. Sciatic nerves containing labeled incorporated materials were removed from animals several hours after injection of their L7 dorsal root ganglia with [3H]leucine and were placed in chambers supplied with oxygen and kept at 38°C for a further period of downflow. The same crest of activity in the nerve was seen and it moved at the same fast rate as had been found in the animal (8). When the nerves in the chambers were exposed to N2, a failure of transport occurred within 15 min of the onset of anoxia. This block of transport was also seen in nerves exposed to NaCN, or to dinitrophenol. These results indicated that fast transport is closely dependent on oxidative phosphorylation (9, 10). The similarity between the time of transport block and the time found for failure of action potentials in mammalian nerves subjected to N2 asphyxiation (10–13) indicated that a common source of ATP is involved. Such a source would supply energy to the sodium pump to maintain the asymmetrical concentrations of K+ and Na+ in the nerve fibers required for excitability and energy to the molecular mechanism responsible for fast axoplasmic transport (10).

In support of this concept, ATP and phosphocreatine concentrations were found to fall and inorganic phosphate (Pi) concentration to rise 15 min after the onset of anoxia (14).

The present study was undertaken to determine how localized the supply of energy is to the transport mechanism. For this purpose, a short length of nerve was made anoxic and the effect of fast axoplasmic transport of such anoxia was measured. By this means it was found that fast transport depends on oxidative metabolism at every small increment all along the length of the fibers. The results were interpreted with respect to a "sliding filament" hypothesis for the mechanism of fast transport (15).

MATERIALS AND METHODS

The methods of injecting the precursor into the cat L7 dorsal root ganglion and preparation of samples are similar to those previously described (6, 10). [3H]Leucine, at a concentration of 5 mCi/ml and at a high specific activity (over 50 Ci/mmol) was supplied as a sterile isotonic saline solution (New England Nuclear Corp.). Approximately 10-μl volumes were injected into the exposed ganglia, and 2 or 3 hr was allowed for downflow into the nerves in vitro. The animals were then killed; the sciatic nerve, L7 ganglion, and dorsal root were removed as rapidly as possible and trimmed free of adhering tissue. The nerves were placed on a strip of filter paper on the bottom of a plastic chamber and a small amount of Krebs-Ringer or lactate-Ringer solution was added in order to keep the nerve moist. The nerves were incubated at 38°C in 95%/5% O2–CO2 3 or 4 hr longer. When a small stretch of nerve was to be made anoxic during in vitro downflow, narrow strips of parafilm coated with petrolatum jelly were placed under and over the nerve, with a sufficient excess of petrolatum jelly present so that the surface of the nerve was covered and oxygen was prevented from entering that region. After a 3 or 4 hr period of downflow, the nerves were removed and the exact boundaries of the anoxic region marked with India ink. The nerves were placed on cardboard strips and dried, and the nerves, including ganglia and dorsal roots, were cut at 3-mm intervals. Each segment was placed in a vial to which 1 ml of Soluene (Packard) was added to solubilize the tissue. Scintillation fluid was added and the activity present in each vial was determined in a Packard Spectrometer.

RESULTS

Transport was blocked by a local region of anoxia placed in the path of the advancing crest of activity. The distance at which the nerve was to be blocked was estimated from the previously determined 400 mm/day rate of fast transport. In the example shown in Fig. 1, the proximal edge of the anoxic region was set 45 mm from the ganglion in a nerve taken from the animal after 2 hr of downflow. A 4-hr period of downflow in vitro was allowed. The control nerve shows a large amount of radioactivity present in the ganglion region that falls to a plateau level distally, then rises to a crest of activity before rapidly falling to baseline amounts. If the distance from the intercept of the front of the crest with the
FIG. 1. 2 hr after injection of a cat L7 dorsal root ganglion with [3H]leucine, the nerve was removed, placed in a chamber, flushed with 95% O2-5% CO2, and kept at 38°C for 4 hr of in vitro transport. A crest of radioactivity had moved down into the control nerve (O) to the position indicated by arrow 3. The opposite nerve (●) was similarly prepared, and in addition covered with petrolatum jelly over a length of 15 mm (starting at a distance of 45 mm from the center of the ganglion) as indicated by arrows 1 and 2 and the hatched bar. Damming was shown by the rise of radioactivity to a peak at the most proximal edge of the anoxic region, falling to baseline level a very short distance distally. The activity present in each 3-mm segment of the control and locally anoxic nerve is presented in cpm on a log scale on the ordinate. The dorsal root, ganglion (G), and sciatic nerve are shown at the distances in mm indicated on the abscissa.

background amount (arrow 3) to the peak of activity at the L7 ganglion, and the total time of 6 hr (2 hr in vivo and 4 hr in vitro) is taken as an estimate of the rate, activity had advanced to full distance expected of fast axoplasmic transport. In the nerve where a local anoxia had been present, the same high amount of activity was seen in the ganglion, as well as the fall to a plateau level. However, at the forward edge of the anoxic region there was an increased amount of radioactivity; just distally inside the anoxic region, a rapid fall to the baseline amount of activity was observed. This represents a damming due to anoxia.

FIG. 2. Local anoxic site placed more distally on the nerve than in Fig. 1. All other symbols are similar to those of Fig. 1 in this and the following figures.

FIG. 3. Time of downflow in the animal 3 hr, in vitro 3 hr. Arrow 2 indicates downflow at the foot of the crest of a control nerve.

The scatter of activity at about 30–50 mm in this and the other examples shown was due to an accumulation of activity in some of the cut ends of nerve branches supplying the hamstrings. These had not been entirely removed, but none of this adventitious accumulation of activity accounts for the damming seen. In later experiments these nerves were trimmed after in vitro downflow. When the anoxic region was placed further down the nerve, these irregularities did not interfere with the pattern of damming.

When the local region of anoxia was placed more distally in the nerve, the peak of dammed activity was smaller, as expected from a reduced time during which materials could flow into the dammed region (Fig. 2). If, instead of allowing downflow for 2 hr, 3 hr was allowed before the animal was killed and the nerve was removed, the pattern of damming above a local anoxic region in vitro was similar (Fig. 3). The region of local anoxia had been set to about 60 mm from the ganglion to take into account the more distal position of the crest at 3 hr.

The damming seen in front of the local anoxic site was similar to that found above a ligated region or above a freeze-blocked region where the fibers had been closed off and downflow prevented (6). The present technique, however, allows a more exact determination to be made of the region of block. There is no mechanical derangement such as occurs after ligation or an indeterminate spread of temperature when freeze-block is used to produce damming. The peak of the increase in activity in the dammed region was close to or at the leading edge of the locally anoxic region; this was seen in all 32 experiments performed. Within the limitations of the sampling size used, this indicates that the effect of anoxia could be identified to within 6 mm, or possibly even 3 mm. The fall of activity just distally inside the anoxic region was remarkably steep, approaching baseline levels within about 6 mm. Very little change in the slope of this fall occurred during the whole of the 3 or 4 hr of local anoxia while the nerve was in the chamber. This adds further evidence to the conclusion previously reached that labeled materials do not diffuse very far in the nerve (5).

The steep fall of activity just inside the local region of anoxia also indicated that an adequate supply of oxygen does
not diffuse into the nerve under the covered zone for more than a few millimeters at most. From the similarity of the amount and pattern of downflow of activity in the nerve in vitro with that found in vivo, we earlier concluded that oxygen can diffuse through most of the radial thickness of the nerve, a distance of about 2–3 mm. From the Krogh-Erlang equation (16, 17), the pressure gradient of oxygen through this tissue appears to be sufficient to maintain oxygenation because of the relatively low oxygen utilization of nerve compared to a more rapidly metabolizing tissue such as muscle. However, it is possible that some of the deeper fibers in thicker sciatic nerves may not be well oxygenated, a point that will require further investigation.

Oxygen diffusion was inadequate when the block (the region of local anoxia) spanned a length over the nerve of only 5–6 mm. Diffusion of oxygen from each end into the locally covered site was insufficient to maintain fast transport, which again suggests that an adequate supply of oxygen does not extend through the tissue for more than about 2–3 mm. However, this type of experimental approach is limited because the actual length of nerve covered by the petrolatum jelly could be in error by at least 0.5–1 mm. Some of the jelly tends to move laterally from the margins of the plastic when gentle pressure is applied to ensure that the surface of the nerve is completely covered.

The failure of transport in the region of anoxia is not due to a rapid closure of the fibers preventing downflow on a mechanical basis. Nerves were prepared for histological examination by means of the freeze-substitution technique to preserve the shape of nerve fibers (18). Under such conditions the fibers were found to maintain a generally cylindrical form during the first several hours of anoxia. At still later times, ovoid formation followed by closure occurs. Such studies will be presented elsewhere. It can be concluded, however, that the fibers were patent and closure does not explain the block.

The reversibility of an anoxic block that produces damming was investigated by placement of petrolatum jelly on nerves for different times followed by its removal for a further period of in vitro downflow. When the local block was removed at times up to 1 hr, fast transport resumed its usual downflow and pattern (Fig. 4). In only a few cases was a small peak of activity found remaining at the blocked region [in these cases, though, there was a broadening of activity distally into the region that had previously been made anoxic (Fig. 5)]. The one obvious effect was the failure of the crest to advance to the same position as the control. This shows that after the period of block of fast transport by local anoxia, fast axoplasmic transport resumes its usual rate of advance.

With local blocks of oxidation over 1 hr in duration, a damming of activity remained present at the front of the asphyxiated region (Fig. 6). There was some broadening of the peak of the dammed activity distally and a slight amount

![Fig. 4](image_url)

**FIG. 4.** Nerves made locally anoxic at the positions indicated by the two bars. One nerve (O) remained covered for all of the 3 hr of in vitro downflow (arrow 1). The covering over the other nerve (●) was removed after 1 hr (arrow 2). Arrow 3 shows the extent of movement of its crest. Arrow 4 and the dashed line indicate the expected downflow of the nerve if it had not been made temporarily anoxic.

![Fig. 5](image_url)

**FIG. 5.** A local block of oxidative metabolism was produced by placing petrolatum jelly on the nerve at the distance from the ganglion indicated by the bar and arrow 1 and removed from the nerve (●) 1 hr later. The crest shown by arrow 2 fell short of the downflow in the control nerve (O), as indicated by arrow 3.

![Fig. 6](image_url)

**FIG. 6.** Petrolatum jelly placed on the nerve (●) with its leading edge at the position shown by arrow 1 and removed after 11/4 hr. The control nerve (O) shows the usual pattern of downflow (arrow 2).
of activity tailing distally in the nerve, which possibly indicates a limited degree of recovery. Such limited recovery, if any, was, however, insufficient to maintain the usual pattern that indicates fast transport.

**DISCUSSION**

The present studies indicate that a local supply of energy derived from oxidative metabolism is required for fast transport at every increment along the length of the axon. From the rapid fall-off of activity in the anoxic region we can infer that neither \( O_2 \) nor the ATP produced by oxidative metabolism in the oxygenated region can spread more than a few millimeters in the fiber. The labeled components also remain in situ. A general mechanism of propulsion of labeled components, such as a peristaltic mechanism, would not account for this result. The evidence fits well with a new model for fast axoplasmic transport, one requiring energy supplied by ATP (15). This model is based on the linearly oriented microtubules and/or neurofilaments that are assumed to play a role in axoplasmic transport (19-21). It differs from other models in that it is designed to account for transport not only of particulates (19), but also of the labeled protein and polypeptides, and even free leucine, that are carried down the fibers (at the same rate) by the fast transport system (1, 5, 7, 22, 23). In this model, a protein "transporting filament" is synthesized in the soma and enters the axons. To it other newly synthesized proteins, polypeptides, and particulates are bound. All these species are then carried down the axon as the transporting filament slides along the stationary microtubules and/or neurofilaments of the axons by means of crossbridges between the transporting filament and the microfilaments and/or neurofilaments. These are presumed to perform the same cycle of ratchet propulsions postulated to underlie the movement of the sliding filaments in contracting striated muscle (24). The crossbridges in the muscle require ATP; our studies showing the close dependence of fast transport on oxidative phosphorylation and ATP (14) fulfills a key requirement of this hypothesis. Actomyosin-like proteins that react with ATP are present in a large number of lower-cell forms that show motility (25-29), as well as in mammalian brain (30) and peripheral nerve (31). These are suggestive findings which require further study.

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