High Voltage Electron Microscopy Studies of Axoplasmic Transport in Neurons:
A Possible Regulatory Role for Divalent Cations

MARK E. STEARNS
Department of Molecular, Cellular, and Developmental Biology, University of Colorado,
Boulder, Colorado 80309

ABSTRACT Light and high voltage electron microscopy (HVEM) procedures have been employed to examine the processes regulating saltatory motion in neurons. Light microscope studies demonstrate that organelle transport occurs by rapid bidirectional saltations along linear pathways in cultured neuroblastoma cells. HVEM stereo images of axons reveal that microtubules (Mts) and organelles are suspended in a continuous latticework of fine microtrabecular filaments and that the Mts and lattice constitute a basic cytoskeletal structure mediating the motion of particles along axons. We propose that particle transport depends on dynamic properties of nonstatic microtrabecular lattice components.

Experiments were initiated to determine the effects of changes in divalent cation concentrations (Ca$^{2+}$ and Mg$^{2+}$) on: (a) the continuation of transport and (b) the corresponding structural properties of the microtrabecular lattice. We discovered that transport continues or is stimulated to a limited extent in cells exposed to small amounts of exogenously supplied Ca$^{2+}$ and Mg$^{2+}$ ions (<0.1 mM). Exposure of neurons to increased dosages of Ca$^{2+}$ and Mg$^{2+}$ (0.2–1.0 mM) stimulates transport for 2–4 min at 37°C, but after a 5- to 20-min exposure the saltatory movements of organelles are observed gradually to become shorter in duration and rate until particle motion ceases to occur. HVEM observations demonstrated that Ca$^{2+}$- and Mg$^{2+}$-stimulated changes in the organization of the microtrabecular lattice are associated with the cessation of motion. Ca$^{2+}$-containing solutions produced contractions of the microtrabecular filaments, whereas Mg$^{2+}$-containing solutions had the opposing effect of stimulating an elongation and assembly (expansion) of microtrabeculae. On the basis of these observations we hypothesize that cycles of Ca$^{2+}$/Mg$^{2+}$-coupled contractions and expansions of the microtrabecular lattice probably regulate organelle motion in nerve cells.

Although it is widely acknowledged that intracellular transport depends on the architectural properties of nonmuscle cell systems, the basic regulatory mechanisms remain enigmatic. The rapid transport of organelles in neurons represents one of the more spectacular examples of cytoplasmic granule movement that has been investigated. In this system, light microscope observations have revealed that organelles in neurons move bidirectionally in a rapid, saltatory manner along linear pathways in the axon (13, 14, 31, 37, 44, 55). Electron microscopy evidence (1, 15, 26, 47, 50) and drug studies (1, 12, 27, 33) have indicated that either axoplasmic microtubules (Mts) or microfilaments (Mfs) probably mediate this particle transport.

It is uncertain, however, whether these filamentous elements, functioning alone or in cooperation, are sufficient to produce the orderly motion of cellular components. Byers (7), for example, has reported that exposure of axons to colchicine, at levels that drastically reduce the numbers of Mts, does not appear to interfere with the continued transport of radioactively labeled material. She interpreted the data to mean that a component other than Mts may be directly involved in the regulation of fast axonal transport. Recent experiments with neurons incubated in high Ca$^{2+}$ solutions (3) have provided added support for her suggestion that Mts are not required for fast transport. The conclusion contradicts several reports in which the use of antimicrotubule drugs has provided convincing evidence that intact Mts are required for fast transport (12,
In fact, several models that directly implicate Mts in the regulation of transport have been proposed (6, 42, 43, 46, 47). The opposite nature of the conclusions reached would indicate that further work is required to resolve the roles of filamentous structures in fast transport.

The main difficulty with the above studies is that the antimiticotic drugs (colchicine, podophyllotoxin, vinblastine, and Ca\textsuperscript{2+}) tend to affect numerous physiological processes unrelated to Mts (10, 16, 17, 52, 53, 56). In addition, attempts to relate mechanisms of transport to the properties of specific structures have been complicated by the fact that neurons contain varying amounts of diverse fibrous components, including Mts, Mfs, neurofilaments (Nfs), myosin, and sulfated mucopolysaccharides from which nerve cells might construct alternative transport systems. Burton and others (6, 27, 35, 40, 51), for example, have found that Mts and Nfs are coated with a fine meshwork of anastomosing filaments. Since the filaments can be stained with alkaline dyes (ruhenium red, lanthanum, and alcin blue) reactive for mucopolysaccharides, the authors have suggested that Ca\textsuperscript{2+}-sensitive contractile properties of these components might regulate fast axonal transport in nerve cells, an intriguing concept that deserves further attention.

High voltage electron microscopy (HVEM) stereo techniques have been developed recently by Porter and colleagues (4, 5, 54) for examining the three-dimensional organization of the cytoplasmic matrix. From thick section studies of nerve tissue they have reported that Mts and organelles are embedded in a latticework of space-filling, anastomosing filaments, termed microtrabeculae (11). HVEM studies of whole-mount pigment cells have further revealed that cycles of pigment granule aggregation and dispersion rely on morphological transformations (contractions and energy-dependent expansions) of the microtrabecular components in which Mts and granules are suspended (8, 39). On the basis of these types of studies, Ellisman and Porter (11) have recently proposed that similar, albeit less dramatic, changes in lattice organization may function to regulate vesicle motion in neurons.

In this paper we have investigated the ultrastructural properties of components associated with organelle transport in cultured neuroblastoma (C1300) cells, used as a model nerve cell system. The studies have been extended to investigate the role of divalent cations (Ca\textsuperscript{2+} and Mg\textsuperscript{2+}) in fast axonal transport. We have examined the correlative effects that exogenously supplied ions can have on both the continuum of transport and the corresponding organizational properties of cytoskeletal components mediating movement. With these results we have constructed a model describing the role of the microtrabecular lattice in fast axonal transport.

**Table I**

| Treatment | Diameters (nm) | Lengths (nm) |
|-----------|---------------|--------------|
| mHBSS     | 5.1 ± 2.2     | 180 ± 22     |
| 0.10 mM Ca\textsuperscript{2+} | 7.5 ± 3.1 | 163 ± 16 |
| 1.0 mM Ca\textsuperscript{2+} | 17.1 ± 6.3 | 93 ± 21 |
| 0.10 mM Mg\textsuperscript{2+} | 6.1 ± 1.1 | 195 ± 31 |
| 2.0 mM Mg\textsuperscript{2+} | 3.3 ± 0.9 | 272 ± 65 |

Summary of measurements of the sizes of microtrabeculae in axons of neurites exposed to exogenously supplied Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions for 20 min at 37°C in mHBSS containing 1 mg/ml A23187. In contrast to control experiments with solutions of 0.1% DMSO, cell behavior and ultrastructural properties were not affected. The pH was routinely adjusted to 7.3 with dilute NaOH or HCl and the osmolality adjusted to ~325 mosM with NaCl.

**HVEM Procedures**

During examination in the light microscope, neurons grown on gold grids were fixed with drops of 2% glutaraldehyde in mHBSS at 37°C for 30 min. Cells were then washed three times with HBS, postfixed with 1% osmium tetroxide for 3 min at 4°C, and washed with HBS. After three rinses in distilled water, cells were dehydrated in a graded acetone series and dried by the critical point method. Cells were lightly coated with a layer of carbon and stored in a vacuum desiccator for up to 2 wk. The neurons were examined under an accelerating voltage of 1,000 kV. The whole-cell fixation procedure used is similar to that described by Wolosewicz and Porter (54).

The sizes of the microtrabeculae were determined from samples experimentally treated for 20 min and fixed for HVEM. The data (Table I) represents...
measurements that were averaged from 100 or more trabeculae examined in three separate samples for each experiment. For these calculations, thin areas of the axon near the base of the growth cone were photographed in stereo at \( \times 25,000 \). The negatives were printed at \( \times 100,000 \), and the diameters and lengths of trabeculae found coating the surfaces of the microtubules were measured in a stereo viewer with a vernier caliper.

**RESULTS**

**Light Microscope and HVEM Correlative Studies**

The motion of organelles (lipid, lysosomal, and smooth endoplasmic reticulum [SER] vesicles) in neurons was followed with time-lapse phase microscopy. Stop-motion analysis of movement of 200 particles showed that they move by rapid saltations (0.5–9 \( \mu \text{m/s} \)) in a bidirectional fashion along axons. Further slow-motion studies of these films indicated that the motion of individual particles is subject to localized control, in that transport usually occurs along relatively linear axoplasmic tracts (Figs. 1 and 2). For example, nearby particles are often observed to move simultaneously in opposing directions along adjacent axoplasmic tracks (Fig. 2). In general, though, a given particle's motion is continuous in one direction for a series of saltation events extending along the length of the axon (Figs. 1 and 2). Many of these organelles accumulate in the growth cone and lamellar regions, where they are seen to continue streaming, albeit in a nonlinear, stochastic fashion. Interestingly, in developing regions of the growth cone, clusters of these particles are sometimes observed suddenly to stop moving and then move en masse to fill newly spread lamellar structures of the growth cone. Light studies of 350 lamellae demonstrated that most (311 or 89%) lamellae are normally active in particle transport, although some lamellae (39) were not involved in organelle motion.

HVEM pictures of fixed and critical-point-dried neurons reveal that the axoplasmic matrix consists primarily of aligned microtubules and a continuous space-filling microtubercular lattice (MTL) in which the Mts and organelles are suspended (Figs. 3–5). Figs. 3–5 show that a fine network of the microtubercular filaments (5.1 nm diameter) fill spaces between the Mts and organelles and serves to interconnect Mts with other Mts and Mts with the suspended organelles in both axonal (Fig. 4) and growth cone regions (Fig. 5) of the cell. When lamellae, or spreading regions of cells, were examined at the ultrastructural level, 280 of 311 (90%) of those known to be involved in organelle transport at the time of fixation contained Mts in close association with the organelles. The 39 lamellae not involved in transport contained a fine meshwork of trabecular filaments, of which only five lamellae (or 13%) contained any recognizable Mts, indicating that microtubules and their associated lattice components are important for organelle transport.

In this regard, we observed that some neurites contain centralized cores of Mts and that the remaining axoplasmic area is filled with the MTL. Light microscope and HVEM studies of these cells demonstrated that the transport of organelles is localized in the regions defined by the Mt bundles and that transport does not occur in regions containing strictly the MTL component.

**Divalent Cation Effects**

Neurons washed in mHBSS containing the ionophoric agents A23187 (1 \( \mu \text{g/ml} \)) or 13 mM NaH2PO4 (2) plus low levels of added Ca2+ and Mg2+ ions (0.05–0.1 mM) did not exhibit significant changes in their cell shape or transport activities over the pH range of 6.8–7.5 tested. If the amount of A23187 used is increased to 5 and 10 \( \mu \text{g/ml} \) concentrations, the cells react by rapidly retracting their extended axons, and HVEM studies show that A23187 at 10 \( \mu \text{g/ml} \) levels produces a contracted lattice. Thus, in all the experiments reported here, 1 \( \mu \text{g/ml} \) A23187 or 13 mM NaH2PO4 was employed in order to minimize the independent effects of these agents, since under these conditions little or no detachment and/or retraction of the neurites occurred. In all the studies reported, identical results were obtained in experiments using either of these ionophoric agents.

Time-lapse studies of the treated cells show that particle motion is stimulated in rate and amount in neurons exposed to Hanks' solutions containing small amounts of either Ca2+ or Mg2+ cations (<0.1 mM) over a pH range of 6.8–7.5. At increased levels of either Ca2+ (0.2–1.0 mM) or Mg2+ ions (0.5–2.0 mM) tested, the ions exert a dose-dependent effect on both cell shape (Fig. 6) and the rates of axonal transport (Table II). We found that the initial influx of these ions into cells stimulates transport, but that, with time, the motion of particles becomes noticeably irregular and jerky, and in many cases motion finally ceases altogether. At even higher Ca2+ levels (2–20 mM), transport invariably came to a rapid halt by 2–5 min. In addition to the effects on transport, Ca2+ induces a retraction of lamellar extensions apparently as a result of contractions of the axoplasmic matrix (compare Fig. 1a–c, h, and i with Fig. 1d–f).

In comparison to the Ca2+ ion effects, high Mg2+ levels (5–20 mM) simulate the formation of lamellae, and cells often exhibit some irregular, sporadic transport activities after 20 min at 37°C. Note that, in very large axons containing numerous mitochondria, some transport still continues in the presence of added Ca2+ or Mg2+, even at the 20-mM levels tested.

**Control Studies**

In control studies, we found that the substitution of other metal ions (Ba2+, Mn2+, Co2+, Sr2+, Ni2+) at 0.5- and 1.0-mM levels for Ca2+ and Mg2+ ions does not have an effect on the continuation of transport. Excess Co2+ and Sr2+ at 10-mM levels has an inhibitory effect on transport. For some experiments, neurons were preincubated in mHBSS containing either Mg2+ or Ca2+ ions at 0.1-mM levels for 1 min, and the cells were immediately transferred to mHBSS solutions containing the opposing divalent ion (Ca2+ or Mg2+) at 1- and 2-mM levels (Table II). In these studies, we observed that the time interval required for transport to slow down or halt in response to added Ca2+ and Mg2+ was approximately doubled from the time periods recorded for experiments in Table II, where the preincubation step was omitted.

Finally, to further convince ourselves that an influx of Ca2+ into the cells was in fact responsible for the results recorded in Table II, neurons were exposed to 10 mM Ca2+ for 5 min in Hanks', and these cells were transferred to mHBSS containing A23187. Transport activities in the neurons came to a halt after relatively short incubation periods of 3–5 min in the A23187 solutions, indicating that membrane-bound Ca2+ was entering the cells. Alternatively, when the cells were preexposed to La3+ ions (1.0 mM) for 5 min, the transport of particles continued unabated when the neurons were subsequently transferred to ionophore solutions containing 1.0 mM Ca2+. The experiments indicate that La3+ ions bind to membrane binding sites specific for Ca2+ transport, preventing A23187-mediated Ca2+ uptake.

For some studies with Mg2+ ion, the Mg2+ ionophore DCCD (10 \( \mu \text{m} \)) was substituted for A23187. Results identical to those reported in Table II using A23187 are obtained from experiments with DCCD.
HVEM Studies of the Divalent Cation Effects

When the neurons were exposed to either low Ca\(^{2+}\) and Mg\(^{2+}\) levels (0.1 mM) or briefly exposed (1 min) to high doses (1-mM levels) of Ca\(^{2+}\) and Mg\(^{2+}\), the ultrastructural organization of the axoplasm generally remained unchanged from that seen in the untreated cells (Figs. 3-5). However, in neurons exposed to increased Ca\(^{2+}\) ion concentrations (0.2-1.0 mM) for
relative long time periods, HVEM studies (Figs. 6-8) show that coincident with retractions of lamellae (Fig. 1) the microtrabecular lattice gradually becomes more coarsely divided. The individual microtrabeculae associated with the Mts (Fig. 8) and organelles (Figs. 7 and 8) become thickened in appearance (17.1 nm) as compared with the diameters of trabeculae in controls (compare Figs. 4 and 5 with Figs. 7 and 8; see Table I). In addition, the intertrabecular spaces simultaneously increased in size to about double their control values. Associated with these changes in trabecular conformation and lattice organization, the axonal microtubules in Ca"-treated cells were often found to align in closely appressed parallel arrays (Fig. 8).

Exogenously added Mg2+ ions (0.5–2 mM) caused neurons gradually to spread and form new lamellipodia. In contrast to the Ca" ion effects, the lattice in these cells was more finely divided and exhibited increased numbers of thin, elongate trabeculae (Table I). The surfaces of both the Mts and particles were coated with numerous thin filaments (Fig. 9). Compare Fig. 9 with Figs. 4 and 5 of controls and Figs. 7 and 8 of Cat+-treated cells. In the newly forming lamellar regions, the trabeculae and bundles of Mts and Mfs appeared in the process of assembly and elongation. The addition of nonspecific metal ions (Ba2+, Sr2+, Co2+, Mn2+, Ni2+, Cu2+) at 1-mM levels in place of Ca2+ and Mg2+ did not have similar effects, indicating that a specific fluctuation of Ca2+ and Mg2+ ions plays an important role for transport-related activities of the lattice. The above Ca2+/Mg2+ experiments were carried out simultaneously on three specimens for each experiment, and the samples were prepared for HVEM under identical conditions to eliminate possible interpretational errors that may arise from the preparative steps.

Divalent Chelator Experiments

In attempts to further determine what importance divalent cations might play in the regulation of transport, neurons were exposed to 1-mM amounts of the cation-chelating agents EDTA and EGTA in the presence of A23187 (see Materials and Methods). From time-lapse studies we found that cells exhibit both an increased amount of ruffling and transport activity during the first 5 min of treatment. After 20 min of continuous exposure, however, the spreading lamellae are essentially frozen in an extended form and organelle transport is either discontinued or at least limited in its extent. In stereo HVEM images, the lattice in the EDTA- or EGTA-treated cells appears coarsened and distorted in appearance, somewhat resembling that in the Ca"-treated cells (Figs. 7 and 8). These changes in lattice organization are especially apparent after the exposure of cells to EGTA solutions for 20 min. Thus, the removal of either free Ca2+ or Mg2+ ions from the axoplasm seems to disturb ion balances necessary for the maintenance of a structurally and functionally sound lattice. When increased amounts of EGTA and EDTA (3 and 10 mM, respectively) are employed in the experiments, the axonal extensions detach from the substrate and rapidly retract into the cell body.

Table III shows time intervals required for the recovery of transportation neurons after the inhibition of transport with 1 mM EGTA and EDTA solutions. In general, we found that transport recovery times are reduced to a minimum, as judged by observations made on at least 10 different cells in each experiment.
FIGURE 3. View of part of an axon showing microfilament bundles (Mf) near the subplasmalemmal surface and numerous microtubules (M) throughout the axoplasmic matrix. V, vesicle. Microtubules and organelles are suspended in a fine cross-linking network of filamentous material, the microtrabecular lattice (arrowheads). An area from Fig. 3 is shown at a higher magnification in stereo in Fig. 4. X 35,000.
preparation, if low levels of Ca$^{2+}$ and Mg$^{2+}$ (0.05 mM) are added to a recovery buffer of mHBSS with 13 mM NaH$_2$PO$_4$. When higher Ca$^{2+}$ and Mg$^{2+}$ (0.2 mM) levels are used in the buffer, recovery times are reduced to 2 min, but transport is subsequently observed to halt by ~5–10 min. HVEM studies reveal that the lattice in these neurons is contracted in Ca$^{2+}$.

**FIGURE 4** Stereo pair showing a three-dimensional array of aligned microtubules (M) suspended in a cross-linking lattice work of trabeculae (arrowheads). V, vesicle. In these thick regions of the axon, a superimposition of structural information makes it difficult to determine organizational patterns of the microtrabeculae networks. For proper viewing of stereos, a stereo viewer should be employed. X 114,000.

**FIGURE 5** Stereo pair showing part of a growth cone region of a neurite. A vesicle (V) is seen in close juxtaposition with microtubules (M). The microtrabeculae (arrowheads) form a prominent continuous lattice-work in this region of the cell and appear to cross-link the surfaces of the particle with the microtubules. X 90,000.
buffers and expanded or gelated in Mg²⁺ buffers. In control experiments, the substitution of Ba²⁺/Mn²⁺/Co³⁺/Sr²⁺ (0.2 mM) for Ca²⁺ and Mg²⁺ ion does not decrease the recovery times required. In fact, the exposure of cells to high levels of Co³⁺/Sr²⁺ (10 mM) or La³⁺ (1 mM) ions inhibits the recovery of transport, again indicating that Ca²⁺ ions are required for axonal transport since these ions are known to block the entry of exogenously supplied Ca²⁺ ions.

**DISCUSSION**

Intracellular motility in neurons depends on properties of an asymmetrically structured cytoskeleton. The results presented in this paper demonstrate that developing axons of neuroblastoma cells consist of parallel arrays of Mts and organelles suspended in a cross-linking lattice of microtrabeculae. Ellisman and Porter (11) have recently reported similar results from HVEM thick section studies of mature rat nerves where they observed that cross-bridging filaments coat the surfaces of Mts, NFs, and vesicles. The data indicate that microtrabeculae in association with Mts or Nfs form a functional cytoskeletal structure for controlling the transport of organelles along axons. We believe that subtle changes in the organization of nonstatic lattice components must mediate saltatory movements along pathways defined by Mts or Nfs.
**Possible Mechanism of Saltatory Transport**

The observations made in this paper from stereo HVEM studies of hundreds of cells have been assimilated in a model in Fig. 10 describing possible mechanisms of particle transport in neurons. We propose that saltatory transport probably relies on cycles of localized, Ca\(^{2+}\)-triggered contractions and Mg\(^{2+}\)-dependent, ATP-requiring expansions of the microtrabecular lattice within Mt- and Nf-defined pathways. The onset of particle motion would occur with Ca\(^{2+}\)-stimulated detachments of trabeculae from the surfaces of organelles, thus releasing the particles so that they are pulled or pushed in the direction of least resistance, this being the direction of Ca\(^{2+}\)-stimulated contractions. Transport is discontinued when a moving particle(s) contacts an organized, expanded region of the lattice, where Mg\(^{2+}\)-induced attachments of the trabeculae serve to temporarily prevent further particle motion. We suspect that the SER and mitochondria operate to release and resequester small amounts of Ca\(^{2+}\) within Mt- and Nf-defined regions of the axoplasm to produce cyclic changes in organization of the lattice. The bidirectional nature of particle motion would reflect an ability of the lattice components to contract and expand in more than one direction along the linear Mt arrays. As the polarity of microtubules in neurons is predominantly unidirectional (24), this property could conceivably exert a polar effect on the overall direction of movement of axoplasmic organelles, since the majority of axonal transport in neurons is mostly away from the perikaryon.

The importance of intermicrotubule bridges or links in motility has been previously recognized in ciliary axonemes (19), and there is recent data showing that filaments will respond to Ca\(^{2+}\) and Mg\(^{2+}\) ions in vitro to bridge axonemal Mt (58). Similar types of links are seen in thin-section images of axoplasmic Mt, although the links tend to span greater distances to interconnect Mt with other Mt, with Nfs, and with organelles (6, 40, 57). On the basis of these types of studies, Metusals and Izzard (40) and Yamada et al. (57) originally envisaged these filaments to comprise a continuous three-dimensional network important for axoplasmic transport. A similar lattice was subsequently described by Burton and Fernandez (6) in crayfish axoplasm, and they suggested that Ca\(^{2+}\)-dependent contractile properties of a meshwork containing mucopolysaccharides may operate to provide the motive force for transport in neurons.

We believe that the filaments seen associated with Mt and Nfs in conventional thin sections make up at least a part of the complex lattice network associated with these structures in the HVEM results reported here. The lattice associated with Mt and Nfs probably constitutes a commonly shared axoplasmic component that is operative in axonal transport in diverse types of neurons. Porter and colleagues (8, 39) have discovered, in HVEM studies of pigmented cells derived from the neural crest, that pigment granules, like axoplasmic organelles, are contained in a continuous network of microtubeculae associated with radial arrays of Mt. In these cells, pigment granule aggregation appears to depend on a synergistic contraction of the lattice components. Subsequent redispersions of granules in turn relies on a ATP-dependent reformation of microtubecular networks (39). They suggested that properties of radially arrayed microtubecular-Mt structures must provide the basic driving force for pigment granule motion.

In this study, attempts to determine whether changes in the organization of the axoplasm might precede and follow the passage of particles through a given region of the axoplasm...
TABLE III

Reactivation of Organelle Motion by Divalent Cations

| Inactivating agent | Reactivating buffer | Recovery time |
|--------------------|---------------------|---------------|
| 1 mM EGTA          | HBSS                | +10 min       |
| 0.05 Ca ± 0.10 Mg  |                     | +5 min        |
| 0.20 Ca ± 0.10 Mg  |                     | +2 min        |
| 0.05 Ca ± 0.2 Mg   |                     | +2 min        |
| 0.05 Ca + 2.0 Mg   |                     | -10 min       |

1 mM EDTA

| Inactivating agent | Reactivating buffer | Recovery time |
|--------------------|---------------------|---------------|
| HBSS               |                     | +10 min       |
| 0.10 Mg ± 0.05 Ca  |                     | +5 min        |
| 0.20 Mg ± 0.05 Ca  |                     | +2 min        |
| 0.20 Mg ± 0.05 Ca  |                     | +2 min        |
| 0.10 Mg + 0.20 Ca  |                     | -5 min        |

Summarizes the effects of exogenous Ca\(^{2+}\) and Mg\(^{2+}\) (mM levels) on the recovery of intracellular transport in neurons where transport was previously stopped using EGTA and EDTA at 1 mM in mHBSS containing 1 μg/ml A23187 for 20 min. Recovery with time is denoted by (+), and (−) means that transport was stopped at the time indicated.

have not yielded consistent results. In studies of the very thin regions of growth cones (<0.5 μm thick), for example, we have occasionally detected a coarsened (contracted) lattice in the direction of particle motion. Difficulties arise in assessing such differences, since the rate of transport is very rapid (1 μm/s) and small changes (contractions) of the lattice are probably sufficient to generate the movement of a particle. In this regard, the relatively slow rate of glutaraldehyde fixation may not permit adequate preservation of subtle organizational changes that occur in the lattice during transport. Thus, in order to learn more about lattice-regulated transport mechanisms, we experimentally manipulated physiological conditions thought to control the structural and functional properties of the lattice.

Divalent Cation Experiments

It is generally acknowledged that the mobilization of Ca\(^{2+}\) ions serves to regulate the activation of motility events ranging from muscle contraction, ameboid motion, and axonemal bending, to axonal transport. In neurons, Ca\(^{2+}\) (32) and ATP (43) depletion experiments have indicated that axonal transport is a Ca\(^{2+}\)-requiring, energy-dependent process. In this connection, Ca\(^{2+}\) was found to be essential for the onset of rapid transport (22) and shown to move bidirectionally with transported proteins (23). Further, Ca\(^{2+}\) concentrations have been localized in axoplasmic SER cisternae (25), and Ca\(^{2+}\)-binding proteins have been isolated from axoplasm (29). The studies have suggested that Ca\(^{2+}\)-loaded compartments could then serve to release and sequester Ca\(^{2+}\) ions to locally control intracellular motility in Mt channels. Ellisman and Porter (11) propose that vesicle release of Ca\(^{2+}\) from one end and sequestering at the other end could regulate the breakage and formation of trabecular cross-linkages mediating their transport.

In this paper, time-lapse results from experimental manipulations of axoplasmic Ca\(^{2+}\) and Mg\(^{2+}\) ratios (using ionophore...
of microtubules, neurofilaments, microfilaments, and other organelles and other cell systems have demonstrated that the contraction of 

Mn\(^{2+}\) or La\(^{3+}\) for Ca\(^{2+}\) and Mg\(^{2+}\) did not have a noticeable plasmic transport events. The substitution of other ions such as Ca\(^{2+}\) and/or Mg\(^{2+}\) levels could indeed strongly influence axonal motility. Chelating agents have indicated that physiological shifts in calcium ion levels (36, 41, 45). In pigment cells, for example, a slight elevation of the cytoplasmic concentration of Ca\(^{2+}\) was found to induce pigment aggregation, whereas reduced Ca\(^{2+}\) levels facilitated granule dispersion (38). In support of this idea, measurements made in this paper show that Ca\(^{2+}\) indeed can induce formation of trabeculae with thicker diameters (17.1 nm) and shorter lengths (93 nm), presumably representing a contracted state for the lattice.

The importance of Mg\(^{2+}\) ions in regulating cell motility is not understood as thoroughly as is the role of Ca\(^{2+}\). In most studies, Mg\(^{2+}\) ions have been found to have a stabilizing effect on Mt systems in vivo (20, 45, 49). Shigenaka et al. (49), for example, have shown that microchondrial and granule motion in the axopodia of Heliozoans is discontinued in the presence of stabilizing levels of Mg\(^{2+}\) (0.07-10 mM). The data presented in this paper have revealed that, after brief Mg\(^{2+}\) starvation of cells, Mg\(^{2+}\) can produce similar effects in neurons by causing the assembly, elongation, and, ultimately, gelation of Mts and Mt-associated lattice components. Porter and Anderson (47) have recently reported that Mg\(^{2+}\) ions (1-nM levels) induce the formation of a finely divided lattice in cultured cells similar to the Mg\(^{2+}\)-lattice observed in neurons. We interpret the results to mean that Mg\(^{2+}\) is normally employed by the cell as a counterion to Ca\(^{2+}\) in order to maintain the lattice at its highest energy level. It is in this expanded state that the lattice has an ability to respond to Ca\(^{2+}\) ion fluxes that trigger the onset of organelle transport. Data presented in Table II support this proposal, showing that preincubation of cells with Ca\(^{2+}\) or Mg\(^{2+}\) ions can retard the effects of other ions.

Conclusions

The mechanism proposed here is in close agreement with several earlier models of axoplasmic transport (30, 44, 48, 49) where the movement of organelles was proposed to occur by ATPase-dependent activities of cross-bridging Mt-associated filaments. We believe that this basic concept of lattice-controlled transport is probably correct since it adequately accounts for several important features of axoplasmic transport which include: the specific association of particles with Mt or NF bundles; the bidirectional, linear nature of saltatory events; and the sensitivity of transport and lattice organization to changes in Ca\(^{2+}\) and Mg\(^{2+}\) ion ratios.

Mt-directed saltatory motion may be augmented by localized contractions of subplasmalemmal networks in neurons. The Ca\(^{2+}\)-sensitive proteins, actin and myosin, have been identified in neurons by use of both fluorescently tagged antibodies (34) and a variety of EM techniques (1, 9, 36) including heavy meromyosin labeling (9). Isenberg et al. (30) and Goldberg et al. (22) have recently shown an inhibition of transport after the microinjection of the antimicrofilament agent DNase I into axons. Indeed, the HVEM images presented here have indicated that Mt bundles comprise part of the subplasmalemmal cytoskeletal network. Furthermore, recent in vitro viscometric studies have indicated that microfilaments can interact with microtubules coated with MAPs (microtubule-associated proteins) (21). It is reasonable to suppose that Ca\(^{2+}\)-dependent interactions of microtubules, MAPs, and actin-myosin proteins could mediate transport in axons. Models that incorporate such lattice components would account for the observations that antimicrotubule and antimicrofilament drugs can both prevent and interfere with transport in neurons (see Fig. 10).

FIGURE 10 A model describing the structural organization of microtubules, neurofilaments, the cross-linking microtrabecular lattice, and other organelles in the axoplasmic matrix. The lattice emerges from the inner plasmalemmal surface to interconnect the surfaces of microtubules, neurofilaments, microfilaments, and other organelles. In response to a localized release of axoplasmic Ca\(^{2+}\) by the smooth endoplasmic reticulum, the thin, cross-linking trabeculae are believed to contract (double arrowheads) to somehow facilitate the saltatory movement of particle(s) in the direction of least resistance. We believe that the Ca\(^{2+}\)-contracted trabeculae may continue to interact with the particles but they do so without forming permanent attachments that would hinder movement. The duration of a saltatory event is limited by the extent of lattice contraction along defined microtubules. When an organelle encounters an expanded region of the lattice, Mg\(^{2+}\)-stimulated elongations and attachments of the trabeculae may momentarily prevent further motion. A Mg\(^{2+}\)-triggered, ATP-dependent reexpansion of trabeculae is proposed to immediately follow a given lattice contraction event. This would take place in preparation for the next contraction cycle upon the resequestering of Ca\(^{2+}\) ions by SER.

or chelating agents) have indicated that physiological shifts in Ca\(^{2+}\) and/or Mg\(^{2+}\) levels could indeed strongly influence axoplasmic transport events. The substitution of other ions such as Mn\(^{2+}\) or La\(^{3+}\) for Ca\(^{2+}\) and Mg\(^{2+}\) did not have a noticeable effect on the ultrastructure or behavior of neurons. Studies in other cell systems have demonstrated that the contraction of cytoplasmic matrices is sensitive to small increases in the free calcium ion levels (36, 41, 45).
I thank Dave Boggs for excellent technical assistance. I am grateful to Dr. Richard McIntosh for critical reading of the manuscript.

The work was supported by a Canadian Muscular Dystrophy Association (MDA) postdoctoral fellowship to M. E. Steam and MDA grant 1533765 to Dr. Keith Porter.

Received for publication 6 July 1981, and in revised form 6 November 1981.

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