MIGRATORY CELL LOCOMOTION VERSUS NERVE AXON ELONGATION

Differences Based on the Effects of Lanthanum Ion

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ABSTRACT

The effects of lanthanum ions (La³⁺) on the locomotion and adhesion of glial cells and elongating nerve axons are reported. La³⁺ increases adhesion of both glia and of nerve growth cones to a plastic substratum. La³⁺ also markedly reduces glia locomotion, but it does not inhibit nerve elongation. Electron-opaque deposits are seen on the cell surface and within cytoplasmic vesicles of glia and nerves cultured in a La³⁺-containing medium. Possible modes of action for La³⁺ are discussed, particularly the possibilities that Ca²⁺ fluxes or Ca²⁺ involvement in adhesion are altered by La³⁺. The results are consistent with the hypothesis that cell migration and nerve axon elongation differ in mechanism, with respect to both adhesive interactions and the activity of microfilament systems.

INTRODUCTION

The regulation and molecular basis of cell motility have been the focus of much investigation. Many cell movements seem to be driven by two basic molecular systems: one involving microtubules (cilia, flagella, axostyles) (27, 29, 41), another based on proteins with some of the properties of actin and of myosin (2, 3, 6, 18, 34). The latter substances presumably act similar to muscle proteins (17, 19) to produce cell locomotion (amoebae, fibroblasts). The regulation of activity in this second class of motile systems may also be similar to muscle, where Ca²⁺ is believed to play the key role in activating myofilament movement. This paper describes the effects of lanthanum ion (La³⁺), an inhibitor of transmembrane Ca²⁺ fluxes, on: (a) the rates of cell locomotion and axon elongation, and (b) cell-substratum adhesion. An interpretation is offered which supports the hypothesis that a basic difference exists between fibroblast-like locomotion and nerve axon elongation with respect to the action of intracellular filament systems and the role of cell-substratum adhesion.

La³⁺ has an affinity for the outer surfaces of cells (9, 22), apparently by binding strongly to negatively charged groups on the cell surface and thereby displacing cations from their binding sites. La³⁺ decreases cation conductance and has been shown to inhibit Ca exchange across synthetic phospholipid and biological membranes (embryonic heart cells, squid axon, smooth muscle) (22, 28, 42, 44, 45). Because of this effect on Ca²⁺ fluxes, La³⁺ has been used to implicate Ca²⁺ in certain biological phenomena. Cardiac and smooth muscle contraction, reversal of ciliary beat in Paramecium, neurotransmitter release, and histamine release are processes in which Ca²⁺ binding to the membrane or Ca²⁺ flux is thought to be important, and La³⁺ inhibition of these processes has been reported (12,
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22, 28, 30, 31, 45). It should also be noted that ameboid movement, another microfilament-driven process, has been reported to be inhibited by La+++ (15).

Ludueña and Wessells (26) have analyzed the microfilament systems of migratory glial cells and elongating nerve axons. Based on filament morphology and distribution, and on differences of the two cell types in locomotory behavior on agar (40), a model has been proposed to explain the locomotory patterns. The model emphasizes the similarities in the growth cone and ruffled membrane organelles, and also states that glial cells may require a firm adhesion to the substratum and a possible contractile or elastic event to advance the cell body (20, 26).

This paper reports that La+++ at concentrations of 0.5–1.0 mM severely reduces the rate of glia locomotion but has no inhibitory effect on axon elongation. Cell-substratum adhesion is also altered by La+++ (26). Cinematographic and electron microscopical procedures are used to monitor the effects of La+++ on cell movements and ultrastructure.

MATERIALS AND METHODS

Cell and Organ Culture

Lumbrosacral dorsal root ganglia were dissected from 8-day chicken embryos and dissociated in trypsin at 37°C (26). After the trypsin was washed out, the cells were resuspended in modified F12 (38) supplemented with 10% fetal calf serum (F12S10) and nerve growth factor (NGF; 26) and plated in 35-mm plastic tissue culture dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) at concentrations of 4 to 20 X 10⁴ cells/dish in a final volume of 2–3 ml of medium. The dishes with cells were placed in a 37°C, humidified incubator for 5–6 h. Ceil-substratum adhesion was measured with the Gall and Boone (14) airblaster method, which uses a repro- after 18–24 h, by washing out the F12S10 medium with several changes of MEM-HEPES. MEM-HEPES was supplemented with dialyzed fetal calf serum and NGF at the same concentrations as in the F12 medium (MEM-HEPES-S10). For some of the organ culture experiments a similar MEM-HEPES was made without Ca++, and fetal calf serum was dialyzed exhaustively against distilled water to remove Ca++. NGF was also prepared without Ca++. In the homogenization salt solution (47).

Rates of Cell Locomotion and Axon Elongation

After 18–24 h incubation of cell cultures, the F12S10 was replaced with the MEM-HEPES-S10 + NGF with LaCl₃. Control dishes received MEM-HEPES-S10 + NGF without LaCl₃. A dish was placed on the stage of a Zeiss inverted microscope, and a field of cells was chosen for measurement of cell locomotion and axon elongation. A picture of the field was taken immediately and then every hour for 8–9 h. (If intervals between pictures are longer than 1 h, then it is difficult to trace individual moving cells.) The dish remained on the stage, and the temperature was maintained at 37°C by a Sage Aircurtain Incubator (Sage Instruments, Div. Orion Research, Cambridge, Mass.). After making 8 X 10 inch prints of the resultant photographs, each cell was identified, and the distance was measured between the position of the nucleus at 0 time and the position at the end of the picture-taking sequence. After taking magnification into account, this distance was used to calculate an augmented diffusion constant, D*, which has been used previously as a measurement of cell motility (13). Axon elongation was determined by measuring axon lengths at 0 time and at the termination, and was expressed in micrometers per hour per axon (as in reference 24).

The prints were used to determine the vectors of the direction of displacement of individual glia between 0 time and 4–5 h and between the latter point and the termination. The angle between these two vectors was measured with a protractor (Sterling 544). This angle was used to test whether glia execute a random walk (13).

Cell-Substratum Adhesion

Cell cultures were changed to MEM-HEPES-S10 + NGF with or without LaCl₃ after overnight incubation in F12S10. Three squares (2.5 mm² each) were marked on the bottom of each dish with an iridectomy knife, and the dishes were put in a 37°C humidified incubator for 5–6 h. Cell-substratum adhesion was measured with the Gail and Boone (14) airblaster method, which uses a repro-
ducible short blast of compressed air to produce shearing forces in the medium. This shear removes varying numbers of cells or axons from the substratum. A dish was removed from the incubator and the number of glia or axons in a square was counted immediately before blasting. After blasting, the number remaining was counted, and the dish was returned to the incubator. Because of differences in adhesion strength, glial cell adhesion and axon adhesion were measured in separate sets of culture dishes. The percentage of cells or axons distracted from the substratum was calculated thusly:

\[
\text{percent distracted} = \frac{\text{initial count} - \text{final count}}{\text{initial count}} \times 100.
\]

**Time-Lapse Cinematography**

Cell cultures in 35-mm dishes were used. Filming was done on a Zeiss inverted microscope using a Bolex 16-mm movie camera controlled by a Sage cinematographic apparatus, and with the dish at 37°C by use of a Sage Aircurtain Incubator. A 0.25 s exposure was used, and the film speed was 2 or 4 frames/min to allow 8–10 h of filming in one session.

**Electron Microscopy**

Cells which were exposed to La+++ were initially grown in F12S10 + NGF for 18–22 h, and the medium was replaced with MEM-HEPES-S10 + NGF with or without 1 mM LaCl3 for 1 h reincubation at 37°C, followed by primary fixation. Primary fixation was for 1 h at 21°C in 2% glutaraldehyde in Sorensen's 0.07 M phosphate buffer with 0.12 M sucrose at pH 7.4. Secondary fixation was in 1% osmium tetroxide in 0.028 M Veronal-HCl pH 7.4 at 0°C for 1 h. Cultures were embedded in Epon, and the cells were sectioned parallel to the substratum surface (39, 47). Some sections were stained with uranyl acetate and lead citrate before observation with a Hitachi HU-11E electron microscope.

**RESULTS**

**Organ Culture**

Pieces of ganglia placed in organ culture in MEM-HEPES-S10 + NGF without LaCl3 show an extensive halo of glia and axons around the explant at 22 h in vitro (Fig. 1). A carpet of glia has migrated away from the explant by this time, and many axons extend out on top of the glia with a few growth cones protruding beyond the outermost glial cells. The axons usually grow in bundles with single or multiple growth cones. Explants in MEM-HEPES-S10 + NGF with 0.5–1.0 mM LaCl3 have axon outgrowth similar in amount and length to the control explants. In contrast to the control results, however, few glial cells migrate from the chunk, so that nearly all axons and growth cones are directly on the plastic, rather than on a sheet of glia (Figs. 2, 3). If La+++ medium is replaced with the control medium after 48 h of La+++ treatment, the glia begin to migrate out from the explant (Fig. 4).

If La+++ acts by inhibiting Ca++ fluxes in these organ cultures, the effect might be dupli-

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**FIGURE 1** A phase-contrast view of the edge of a living spinal ganglion explant in control medium. Here, at 22 h, many glial cells (G) and nerve axon bundles (N) have migrated out on the substratum. X 180.

**FIGURE 2** A similar view of a ganglion explant cultured in the presence of 0.5 mM La++. Here, at 22 h, abundant axonal outgrowth (N) is evident, but glia have failed to migrate away from the explant. X 180.

**FIGURE 3** The periphery of another ganglion explant cultured in the presence of 1.0 mM La++, and seen here at 42 h of incubation. No glial cells are evident between the axons (N). X 180.

**FIGURE 4** The edge of a ganglion explant that had been cultured for 48 h in the presence of 0.5 mM La++, at which time axons similar to those seen in Fig. 3 were present (extending downward in the picture), and then cultured for an additional 48 h in F12S10 + NGF lacking La++. During this second phase, many glial cells (G) have migrated out among the axons (N). X 180.

**FIGURE 5** A portion of the periphery of a ganglion explant cultured for 48 h in the absence of added Ca++. Though many axonal bundles (N) have extended out, relatively few glial cells (G) have moved away from the explant. There is some hint that those glia which have succeeded in moving from the main tissue mass are associated with axons, and not the plastic substratum. X 180.
cated by growing ganglia quarters in a Ca++-free medium. Before being cultured in the Ca++-free medium, ganglia quarters were washed in Ca++-Mg++-free buffer for 20 min. Most explants attach to the dish, and axons grow out from the tissue masses. As in the La+++-treated cultures, a few glia are seen interspersed among the axons, but there is no carpet of glia surrounding the tissue (Fig. 5). Although there was no Ca++ in the medium, and the quarters were washed in Ca++-Mg++-free buffer, intracellular Ca++ may have remained in the cells throughout this process, so that the cultures may have had a low concentration of Ca++. Atomic absorption spectrometry could precisely determine the concentration of Ca++. 

Cell Motility and Axon Elongation Rates

The organ culture experiments suggested that La++ may be reducing cell motility, but not axon elongation. Gail and Boone (13) have reported that fibroblast cell movement can be described by a random walk model, in which cell motility is expressed with an augmented diffusion constant.

Gail and Boone’s methods (13) were used to test whether glial movement conforms to a random walk model. The intersegmental angles between successive vectors of glial movement (4-5-h periods for each vector) appeared to be randomly distributed. These angles were within the range consistent with an hypothesis of equiprobable intersegmental angles by use of the Z statistic (13). Therefore, glial movement can be described by the random walk model, and motility can be expressed with the augmented diffusion constant.

Glial motility was measured using this technique (Table I). Axon elongation was also determined (Table II). The results indicate that the rate of glial movement in the presence of La+++ is significantly lower than the rate in control medium. However, axon elongation is not inhibited at the same LaCl3 concentration.

Adhesion

Previous studies (14) have suggested that cell motility varies inversely with cell-substratum adhesion. Therefore, cell-substratum adhesion was measured to determine if La+++ inhibition of glial locomotion correlates with La+++ effects on adhesion. The results are presented in Table III. Glia-plastic adhesion is stronger in the presence of 1 mM LaCl3 than in control medium. The difference in adhesion is shown to be statistically significant by use of the Mann-Whitney U test (as in reference 14).

For nerve cells, growth cone-substratum adhesion was measured, but not adhesion of the axon itself to the substratum. The growth cone appears to be a crucially important component in axon elongation, since it is the usual site of microspike movement, and is where new axon material is thought to be added (5, 7, 8). Often a nerve cell will be attached to the substratum only at its cell body and at the growth cone; this is easily seen by moving the dish, since the axon moves as if it is loose, but taut in the fluid.

**Table I**

**The Effect of 1 mM LaCl3 on Glial Cell Motility**

<table>
<thead>
<tr>
<th>MEM-HEPES-S10 + 1 mM LaCl3</th>
<th>MEM-HEPES-S10</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of cells</td>
<td>(T²) µm²</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>20</td>
<td>196</td>
</tr>
<tr>
<td>24</td>
<td>89</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
</tr>
</tbody>
</table>

(T²) is a graphical estimate of the mean square displacement obtained by plotting observed mean square displacement against time (see reference 3). D* is the augmented diffusion constant described by Gail and Boone (13) and is calculated from the equation: \( \langle T² \rangle = 4D^* (t - t^*) \), where \( \langle T² \rangle \) is the square displacement in time \( t \), and \( t^* \) is derived from the plotting of \( \langle T² \rangle \) vs. \( t \). The difference in motility of glia in each experiment is significant at the \( P = 0.01 \) level (by use of the \( F \) test as explained in reference 14). Pooling the data from all experiments shows that the difference is significant at the \( P = 0.001 \) level.
medium (25). Growth cones are not contact inhibited by glial cells, and often move over the upper surfaces of glia. Since glia are a different substratum than the plastic of the dish, adhesion of growth cones to glia was measured independently of growth cone-plastic adhesion (Table III).

Growth cone-plastic adhesion is strikingly stronger in La\(^{+++}\)-containing medium than in La\(^{+++}\)-free medium. Also apparent, (Table III), is the stronger adhesivity of growth cones to glia than to the plastic in La\(^{+++}\)-free medium. The adhesivity of growth cones to glia is as strong in La\(^{+++}\)-medium as in control medium; however, due to the increased growth cone-plastic adhesion in La\(^{+++}\)-medium, there is no statistically

### Table II

**The Effect of 1 mM LaCl\(_3\) on Axon Elongation**

<table>
<thead>
<tr>
<th>MEM-HEPES-S10 + 1 mM LaCl(_3)</th>
<th>MEM-HEPES-S10</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of axons</td>
<td>(\mu)m/h/axon</td>
</tr>
<tr>
<td>11</td>
<td>22.1</td>
</tr>
<tr>
<td>6</td>
<td>17.9</td>
</tr>
<tr>
<td>2</td>
<td>17.4</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
</tr>
</tbody>
</table>

The differences in the rate of axon elongation in these two conditions are not statistically significant.

### Table III

**Cell-Substratum Adhesion**

<table>
<thead>
<tr>
<th>Glial cells on plastic substratum</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of squares</td>
</tr>
<tr>
<td>MEM-HEPES-S10</td>
</tr>
<tr>
<td>MEM-HEPES-S10 + 1mM LaCl(_3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth cones on glial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM-HEPES-S10</td>
</tr>
<tr>
<td>MEM-HEPES-S10 + 1mM LaCl(_3)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth cones on plastic substratum</th>
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</thead>
<tbody>
<tr>
<td>MEM-HEPES-S10</td>
</tr>
<tr>
<td>MEM-HEPES-S10 + 1mM LaCl(_3)</td>
</tr>
</tbody>
</table>

Gliol cell-plastic adhesion was assayed at a blasting distance of 2.2 cm and an air flow rate of 4 liters/min. Growth cone-substratum adhesion was assayed at a blasting distance of 2.2 cm and a flow rate of 2.9 liters/min. Duration of blast in all cases was 0.085 s (see reference 14 for details of air-blast method).

The difference between glia-plastic adhesion in the presence of LaCl\(_3\) and without LaCl\(_3\) is significant at the \(P = 0.025\) level by use of a one-sided Mann-Whitney U Test. The difference between growth cone-plastic adhesion in the presence of LaCl\(_3\) and without LaCl\(_3\) is significant at the \(P = 0.005\) level by use of the same test. The difference between growth cone-plastic and growth cone-glia adhesion in La\(^{+++}\)-free medium is significant at the \(P = 0.01\) level. The difference between growth cone-glia adhesions in the presence and absence of La\(^{+++}\), and the difference between growth cone-plastic and growth cone-glia adhesion in La\(^{+++}\)-medium are not significant.

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significant difference between growth cone-glia adhesion and growth cone-plastic adhesion in the presence of La++. Therefore, the major effect of La++ on growth cone adhesion appears to be an increase in growth cone-plastic adhesivity. Additionally, the impression was gained that La++ increased the fraction of axons attached to the plastic dish after a test blast, though measurements of axon-substratum adhesion were not made.

**Electron Microscopy**

The techniques used to demonstrate La+++ binding with the electron microscope involve primary fixation of cells that had been in La+++ containing medium and washed briefly. The procedure of Langer and Frank (22), in which La+++ was added after primary fixation, failed to reveal many deposits on the glial or nerve cells. Severe contraction of cells, which Langer and Frank reported to occur when glutaraldehyde was added to cells previously immersed in La+++ medium, did not occur using the procedure described in the Materials and Methods section. It was not directly established that the electron-opaque deposits described below truly contain La+++; they are, of course, only present in cultures treated with La+++ before fixation. Electron microprobe analysis could provide a direct demonstration of the presence of La+++ in the deposits.

Attention was focused on visualization of La+++ at the cell surface and within the cytoplasm and on any morphological alterations of intracellular filament systems in La+++ treated cells. Electron-opaque deposits or precipitates with a varied form are seen on or near the plasma membrane and within vesicles, located near the cell surface. Tangential sections of the cell surface, which include the plasma membrane and adjacent micro-exudate material, reveal scattered electron-opaque deposits (Figs. 6, 7, 8, 9, 10, 11). These deposits are not uniformly distributed or exceedingly thick (see reference 22), but are irregularly spaced as discrete units (Figs. 9, 10). The electron-opaque deposits are not seen in the ground cytoplasm, though they are observed within smooth, membrane-bound vesicles (Figs. 7, 8, 10, 11). The smooth vesicles may have been inpocketings of the plasma membrane, that appear to be intracellular, because of the plane of section (Figs. 8, 11).

**Microcinematography**

Cinematographic observations of ruffled membrane and microspike activity of nerve and glia cells provide further evidence of La+++ effects. As reported for fibroblasts (1), the upper surface of glial cells is the site of intense ruffling and undulatory activity. Ruffles appear as the margin of the cell and move centripetally to subside in the region over the middle of the cell. In addition, there is advancement of the cell margin, sometimes followed by retraction of the tail of the cell, resulting in movement across the substratum. Cells in La+++ medium appear to undergo similar types of cell surface ruffling activity. Ruffles and forward advancement of the cell margin are observed, but many cells do not exhibit the phases of elastic recoil or contraction and deadhesion of the tail, characteristic of moving cells. On the other hand, nerve growth cone and microspike activity is not diminished by La+++ treatment. Microspikes extend, bend, and wave about in La+++ medium, just as they do in control medium. In addition, it appeared that axons are more adhesive to the plastic substratum in La+++ medium; that is, axons do not grow in straight lines as they do in F12S10, but are curved or bent after the growth cone has proceeded onward (as in reference 25).

Other cells were changed to Ca++-free MEM-HEPES-S10 with 1 mM EGTA, a specific chelator of Ca++. Glia show much ruffled membrane activity, and nerve growth cone activity is quite normal under such conditions. Extension of the leading edge of glia is seen, but over a total of 9 h of filming, no translocations of cells with typical tail-shortening were observed.

**DISCUSSION**

The major results presented in this paper are:
(a) LaCl₃ at concentrations of 0.5–1.0 mM

Sections of control cells grown in La+++ free medium and exposed to MEM-HEPES-S10 without LaCl₃ show no electron-opaque deposits at either the cell surface or within vesicles (Figs. 12, 13).

The microfilament systems of nerve cells and glia appear morphologically unaffected by La+++ treatment (Figs. 13, 14). The microfilament sheath bundles found near the cell surface of the flattened glia are present, as is the microfilament lattice within both glia and growth cones.
A tangential view near the lower surface of a glial cell that had been incubated for 1 h in La++. Electron-opaque deposits (arrows) are seen in regions cut through the cell surface. Frequently, the deposits are seen just beyond the cell surface in areas where the cell surface is out of the plane of section or in areas where microexudate may have been deposited in the extracellular space beneath the cell (ES). The sheath (99) microfilaments (S) are normal in appearance as are the other intracellular organelles. Microtubule, M; mitochondrion, m. \( \times 23,000 \).

Figures 7 and 8  Electron-opaque deposits (arrows) within vesicular elements of a La+++-treated cell. \( \times 54,000 \).

decreases the migration of glial cells away from explants of dorsal root ganglia, but does not reduce nerve axon elongation.

(6) Similar results are obtained when explants are cultured in medium without added Ca++.  

(c) 1 mM LaCl₃ significantly reduces the rate of locomotion of single glial cells, but has no inhibitory effect on single axon elongation.

(d) 1 mM LaCl₃ increases the adhesion of glial cells and of nerve growth cones to plastic.

(e) With the electron microscope, electron-opaque deposits are seen on the outer cell surface and within smooth vesicles near the surface of nerves and glia immersed in La+++-medium. Gail and Boone (14) have shown that increased adhesion to the substratum correlates with a decreased rate of locomotion by 3T3 cells. Our finding that La+++ induces an increase in glia-to-plastic adhesion may account, at least partially, for the reduction in glia motility. Gail and
Boone’s data indicate that a relatively large decrease in cell motility accompanies a large increase in cell-to-substratum adhesivity. Our data for glia show a large decrease of motility accompanied by a small but statistically significant increase of cell-substratum adhesion.

Although the high locomotory rate of 3T3 cells precludes direct comparison with the current data on glia, the differences in the two systems argue against a simple inverse linear relationship between cell motility and cell adhesion. On the other hand, it is possible that the large decrease in cell motility caused by La++++ might be due to the small increase in adhesion plus La++++ effects elsewhere in the cells.

The measurements of growth cone adhesion and of nerve elongation rates indicate that the adhesion-motility relationship defined for fibroblasts (14) does not hold for nerve growth. Microcinematography indicates that prolonged adhesion of a portion of the growth cone to the substratum need not retard axon elongation, if extension and addition of new material occur elsewhere on the growth cone surface. This observation, plus the fact that La++++ increases growth cone adhesion greatly, but fails to decrease axonal elongation, implies that the relative ease with which growth cone-substratum adhesions can be broken is not crucial to nerve elongation. In contrast, our data and that of Gail and Boone (14) emphasize the need to break cell-to-substratum adhesion, if net migratory cell locomotion is to proceed.

This difference in the importance of deadhesion complements the observation (40), that glia do not spread or migrate in an agar matrix, whereas axons elongate in agar. Thus, in accordance with Gail and Boone (14), it can be hypothesized that there is a range of adhesivity in which cell locomotion can occur: too little or too much is inhibitory. For nerve, the range is broader, at least at the lower end, so that elongation can take place under conditions that preclude cell locomotion. The latter fact is in turn consistent with the idea that axon elongation is dependent upon the extension phase of the locomotory cycle (26, 46) plus net addition of new surface material (5), whereas cell locomotion...
includes both extension and contraction components (26).

The La+++ effects on cell locomotion can be interpreted in terms of an inhibition of transmembrane Ca++ fluxes. Ca++ is the regulatory ion that triggers all types of muscle contraction (10, 16, 21, 35). Calcium may be derived intracellularly from the sarcoplasmic reticulum in skeletal muscle, and extracellularly for cardiac and smooth muscle (10, 11, 16, 21, 22, 37). La+++ inhibits contraction of the latter two muscle types (21, 22, 45); however, it has little effect on the twitch tension of skeletal muscle (21). Some evidence suggests that the plasma membrane of smooth muscle, erythrocytes, and chick embryo fibroblasts has a Ca++ pumping activity analogous to that of the sarcoplasmic reticulum (11, 16, 23, 32). If such Ca++ pumping is a component of cell locomotion, and if La+++ interferes with this process, then a plausible explanation can be offered for the effect of La+++ on cell locomotion.

We have been unsuccessful at measuring directly La+++ effects on transmembrane 45Ca++ fluxes. Insufficient numbers of cells apparently
was responsible for such low uptake that interpretable results could not be obtained.

The fact that La+++ does not inhibit nerve elongation, where a possible Ca++-dependent set of microfilaments is not seen (26), suggests that the activities of the growth cone and microspikes, which contain the distinctive microfilament lattice, do not include contractile events triggered by extracellular Ca++. Extracellular Ca++ is also not required for ruffling membrane activity by glia, though net cell movement from explanted ganglia pieces and translocation as judged by microcinematography do not go on without Ca++ present in extracellular spaces. The continued glial ruffling and growth cone activity observed in Ca++-free medium again agrees with the hypothesis that separates the extension from the “contractile” phases of the locomotory cycle.

An alternative explanation of the role of La+++ in affecting cell locomotion and adhesion is that the ion is involved, like Ca++, in the adhesion process (see reference 43 for review). It is possible that the increase in growth cone and glial cell adhesion is a function of the trivalent ion La+++ substituting for Ca+++ in the adhesion process.

The electron micrographs of La+++ treated cells deserve comment. The apparent electron-opaque La+++ deposits or precipitates on the outer membranes of both glia and nerves are evidence of La+++ binding to the cell coat. In contrast to previous work (22), the deposits do not form a thick blanket next to the plasma membrane. Instead, the pattern of deposition resembles more closely the irregular distribution of histocompatibility antigens visualized by binding of ferritin-labeled antibodies (36), or the particles seen in fracture planes of freeze-cleaved cell membranes (33). It has been reported that La+++ binds selectively to sialic acid residues of cell surface glycoproteins and glycolipids (4). Whether the deposits we see reflect such specific binding is unknown; however, it is interesting to consider the possibility that the deposits represent La+++ binding to some of the normal cell surface binding sites of Ca++.

We express thanks to our colleagues, Brian Spooner and John F. Ash, for critically reading this manuscript. We are also grateful for the valuable technical assistance of Joan T. Wrenn and Belen Sosa.

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REFERENCES


FIGURE 11 Surface-associated electron-opaque deposits (arrow) on a nerve cell body. The vesicle on the right may be continuous with extracellular space. Also note the chainlike shape of the presumed La+++ deposits in this figure and previous figures. × 49,000.

FIGURE 12 A tangential section through the lower surface of the edge of a glial cell in control medium. The plasma membrane (P) and portions of the microfilamentous lattice (L) are seen, but no electron-opaque deposits similar to those seen on La+++ treated cells are evident. × 39,000.

FIGURE 13 The lateral surface of a glial cell fixed from control medium. No electron-opaque deposits like those seen on La+++ treated cell can be found on the tangentially sectioned cell surface, and in the strings of smooth-walled vesicles (V), or in the narrow vesicular channels (E) that run parallel to microtubules (M). Sheath microfilaments, S; intermediate-sized filament, T. × 39,000.

FIGURE 14 A survey view of a glial cell fixed from control medium showing absence of electron-opaque deposits from the lateral and the lower cell surfaces (P). Bundles of sheath microfilaments (S), like these, are seen in both control and La+++ treated cells. × 17,000.


