Histol Histopath (1988) 3: 195-202

Histology and Histopathology

Colchicine causes intrasomatic neurofilament bundles in the mesencephalic trigeminal nucleus and intradendritic bundles in other brain regions

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Summary. The effect of a single intracerebroventricular injection of colchicine on the distribution of organelles in neurons of the mesencephalic nucleus of the trigeminal nerve, the inferior colliculus and the deep cerebellar nuclei was studied. In the mesencephalic nucleus of the trigeminal nerve colchicine produced a dramatic accumulation of neurofilament bundles in the soma of these neurons and did not produce a reduction in the number of lysosomes. In other neuronal populations studied, colchicine produced neurofilament bundles in the dendrites and a reduction of lysosomes from the soma of neurons.

Key words: Neurofilaments, Mesencephalic trigeminal nucleus, Deep cerebellar nuclei, Inferior colliculus, Rat

Introduction

The use of colchicine in neuroscience has focused primarily on its property to inhibit microtubule polymerization, blocking axoplasmic transport (See Dustin, 1984) as well as on its selective toxicity toward granule and other cell types (Goldschmidt and Steward, 1982; Contestabile and Villani, 1984). In previous studies we indicated that colchicine may unmask a unique transport system in the dendrites of neurons (Gorenstein and Ribak, 1985). Light microscopic studies indicated that, in many neuronal populations, lysosomal enzymes, normally found in neuronal somata, were rapidly transported into the dendrites following a single injection of colchicine. Both qualitative and quantitative electron microscopic findings demonstrated that the basis for this enzyme redistribution was an actual translocation of lysosomes into dendrites (Gorenstein and Ribak, 1985; Gorenstein et al., 1988). These studies were conducted in a number of brain regions and revealed an interesting exception to this transport phenomenon. At the light microscopic level, the distribution of lysosomal enzymes in the mesencephalic trigeminal nucleus (MTN) was refractory to the effects of colchicine.

The present study was undertaken to compare the cytological and ultrastructural changes of neurons in the MTN, the deep cerebellar nuclei and the inferior colliculus following colchicine treatment. Are the microtubules in the somata of these neurons disrupted or are they insensitive to this treatment? Also, an analysis of such neurons may provide clues for understanding the mechanism by which lysosomes selectively enter dendrites and not axons.

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Materials and methods

Male Sprague-Dawley rats (200-250 gm) were deeply anesthetized with an intraperitoneal injection of pentobarbital, placed in a stereotaxic apparatus and injected with 100 μg of colchicine in a volume of 10 μl in the left lateral cerebral ventricle. Control animals received an equal volume of saline. Animals were sacrificed twenty-four hours later by intracardial perfusion with 1% paraformaldehyde - 1% glutaraldehyde fixative in 50 mM sodium phosphate buffer, pH 7.0. The brains were removed, cut sagittally along the midline and glued to a Vibratome stage with cyanoacrylate glue. A 3% solution of low-temperature agarose (FMC) at 37° was poured over the brain and allowed to solidify at room temperature. Sagittal sections, 50 μm thick, were cut in ice cold 0.1 M phosphate buffer with a Vibratome. To ascertain the effect of colchicine at the light microscope level, one series of sections was stained to visualize dipeptidylaminopeptidase II (Dpp II) or acid phosphatase as described previously (Gorestein et al., 1985). Another parallel series of sections, stained with cresyl violet, was used to localize and identify areas to be examined at the

electron microscopic level.

Small wedges containing areas of interest were washed in several changes of 0.1 M phosphate buffer, post-fixed in buffered 2% osmium tetroxide for 1-2 hours, dehydrated in an ascending series of ethanols, infiltrated with Araldite and polymerized at 60°C for 72 hours. Blocks were sectioned with a diamond knife at 60-70 nm, using a Reichert ultramicrotome. The ultrathin sections were collected on Formvar-coated 1 x 2 mm single slot grids, stained lightly with Reynold's lead citrate and uranyl acetate, then examined and photographed with a Philips 300 transmission electron microscope at 80 KV.

Results

The mesencephalic trigeminal nucleus (MTN) was identified in control and colchicine injected preparations of the midbrain by the presence of a group of large, spherical neuronal somata with dispersed Nissl substance. These neurons stained intensely for Dpp II and acid phosphatase and were similar in appearance to the large dorsal root ganglion cells.

Control MTN

Neuronal somata of control MTN preparations in the electron microscope displayed a homogeneous distribution of organelles (Fig. 1). Thus, granular endoplasmic reticulum, the Golgi complex, mitochondria and lysosomes were evenly distributed throughout the somal cytoplasm. These large organelles did not display a preferential somal location and appeared randomly distributed (Fig. 2). The smaller organelles, such as vesicles, microtubules and neurofilaments also appeared to be randomly distributed in the somal cytoplasm (Fig. 3). Although some microtubules were found adjacent to mitochondria and lysosomes, these associations did not appear frequently and thus, it is not clear whether they were functionally important. In contrast, microtubules were often found oriented perpendicular to clusters of neurofilaments, suggesting a functional association (Fig. 3).

Colchicine Preparations of MTN

The light microscopic appearance of neurons in the MTN was not significantly changed in the colchicine treated preparations. Although the Nissl stained structures which are normally scattered in the perikaryal cytoplasm were clumped around the nucleus and the plasma membrane, the sizes and shapes of these neurons as well as the histochemical distribution of two lysosomal enzymes, Dpp II and acid phosphatase, did not differ from controls.

The electron microscopic features of these neurons were analyzed for morphological differences. As was observed in our previous light microscopic studies, no changes in the pattern of lysosomal enzyme staining following colchicine treatment were observed in the MTN; the lysosomes in the perikaryal cytoplasm of MTN neurons were unchanged in their relative numbers (Figs. 4 and 5). By contrast, other organelles were significantly altered in their morphology and distribution.

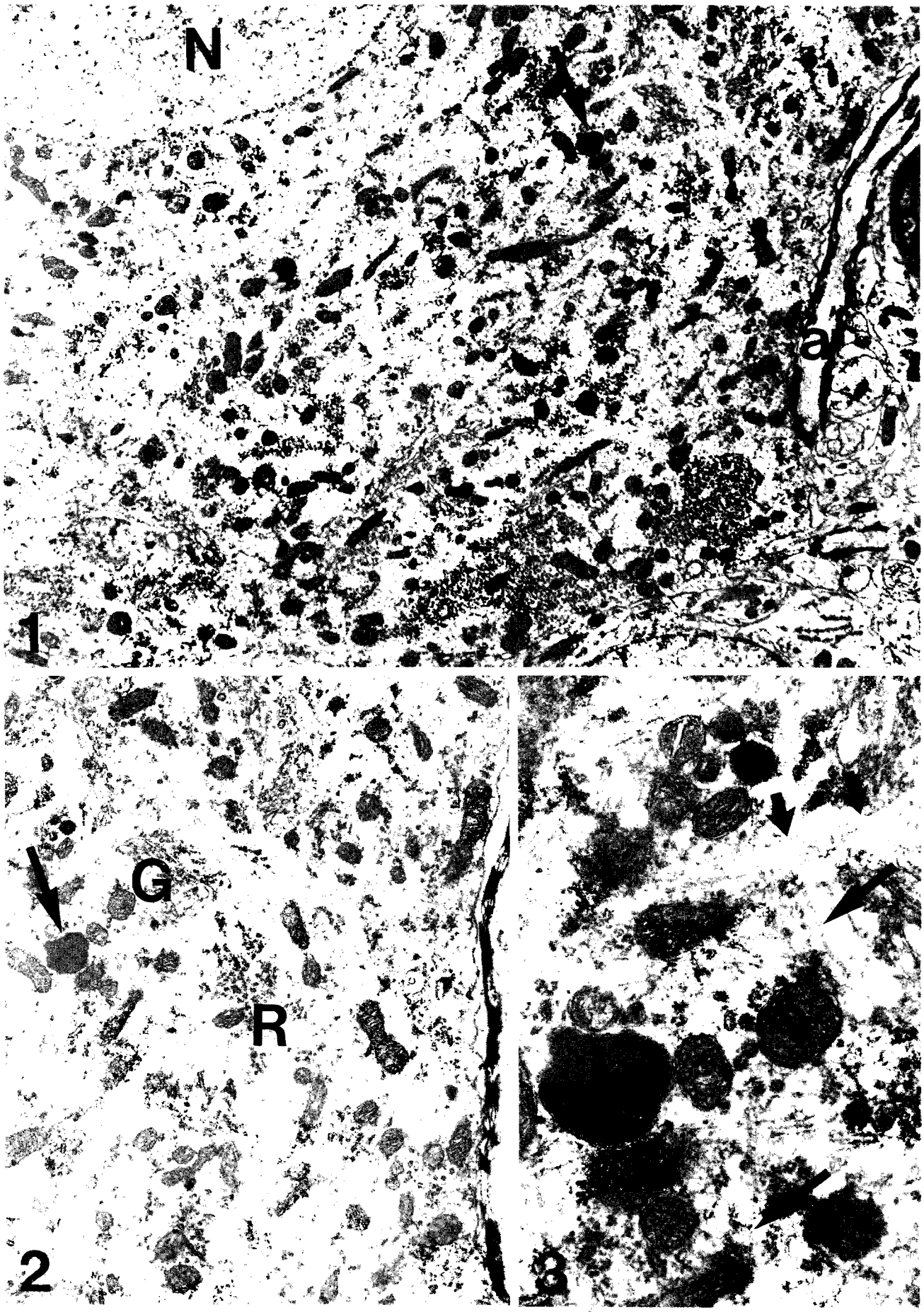
The most apparent morphological alteration in these neurons involved the distribution of neurofilaments. More striking in some neurons than others were the large numbers of neurofilament bundles occupying vast areas of the perikaryal cytoplasm (Figs. 4-9). They were composed of at least ten or more parallel-oriented neurofilaments (Figs. 5-7). Often, many of these bundles were oriented parallel to the nuclear envelope (Fig. 4). Some bundles formed crystal-like structures where the neurofilaments within the bundle were interconnected by crossbridges (Figs. 7 and 9). Microtubules and other organelles appeared to be excluded and were never found within these bundles.

Other organelles were also affected by the colchicine treatment. Microtubules were rarely observed if at all in these preparations. Another interesting alteration was the association of the cisternae of the granular endoplasmic reticulum and many polyribosomes with the nuclear envelope (Figs. 4 and 6). Although they were packed tightly against the nuclear envelope, these cisternae failed to form the distinct parallel structures that are recognized as Nissl bodies.

Other Brain Regions

In previous ultrastructural analyses of the olfactory bulb and facial nucleus from colchicine treated animals (Gorenstein and Ribak, 1985; Gorenstein et al., 1988), neurons which possessed dendrites displayed smaller numbers of lysosomes in the perikaryal cytoplasm and greater numbers in their dendrites. These basic results were confirmed in the present electron microscopic preparations of the deep cerebellar nuclei and the inferior colliculus. The somata of deep cerebellar nuclei neurons displayed normal features in the non-treated preparations (Fig. 10). Symmetric axosomatic synapses were commonly observed as well as the typical distribution of organelles (Fig. 10). However, the somata from the colchicine preparations showed a more watery cytoplasm and a lack of lysosomes and cisternae of granular endoplasmic reticulum (Fig. 11). In contrast to the MTN neuronal somata, we did not observe bundles of neurofilaments in these neuronal populations. The dendrites of these neurons and those in the inferior colliculus, however, often exhibited some neurofilament bundling (Fig. 12).

Figs. 1-3. Electron micrographs of a neuronal soma in the MTN from a non-treated preparation. **Fig. 1** shows the nucleus (N) and a portion of the perikaryal cytoplasm of this soma. The organelles, including lysosomes (arrow) are homogeneously distributed. A myelinated axon (a) lies outside the soma. × 8,600. **Fig. 2** shows an enlargement of this soma with the same lysosome (arrow) as that found in **Fig. 1.** Note the random distribution of the mitochondria, Golgi complex (G) and the polyribosomes (R). × 13,000. **Fig. 3** shows a further enlargement from the area around the lysosome (L) found in the two previous figures. Note the presence of microtubules (large arrows) and neurofilaments (small arrows). × 40,000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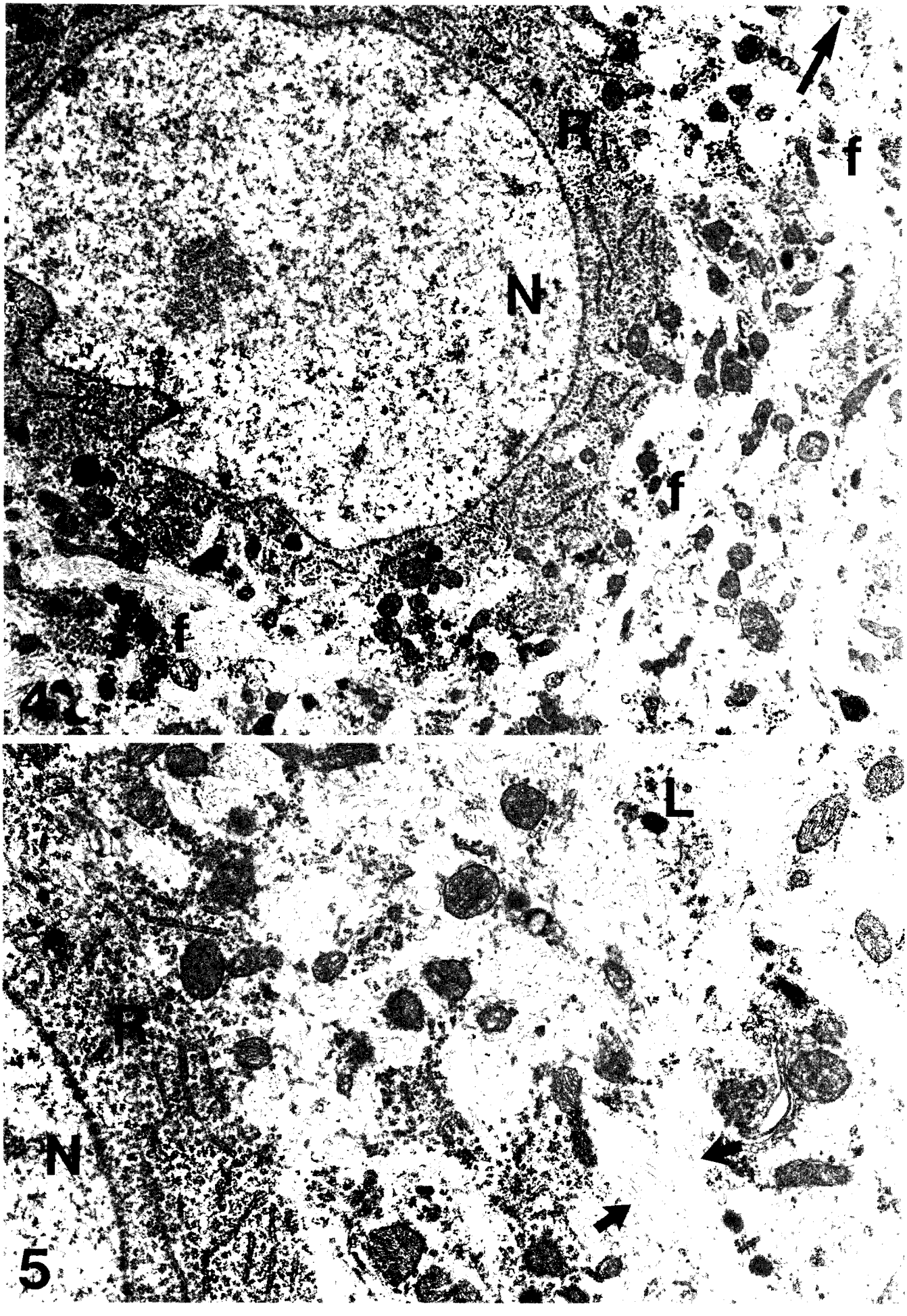
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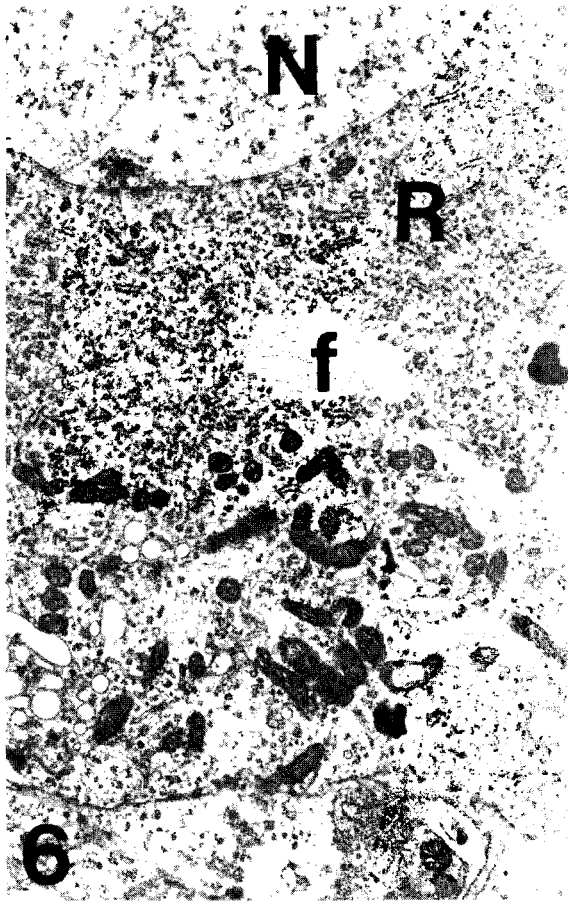
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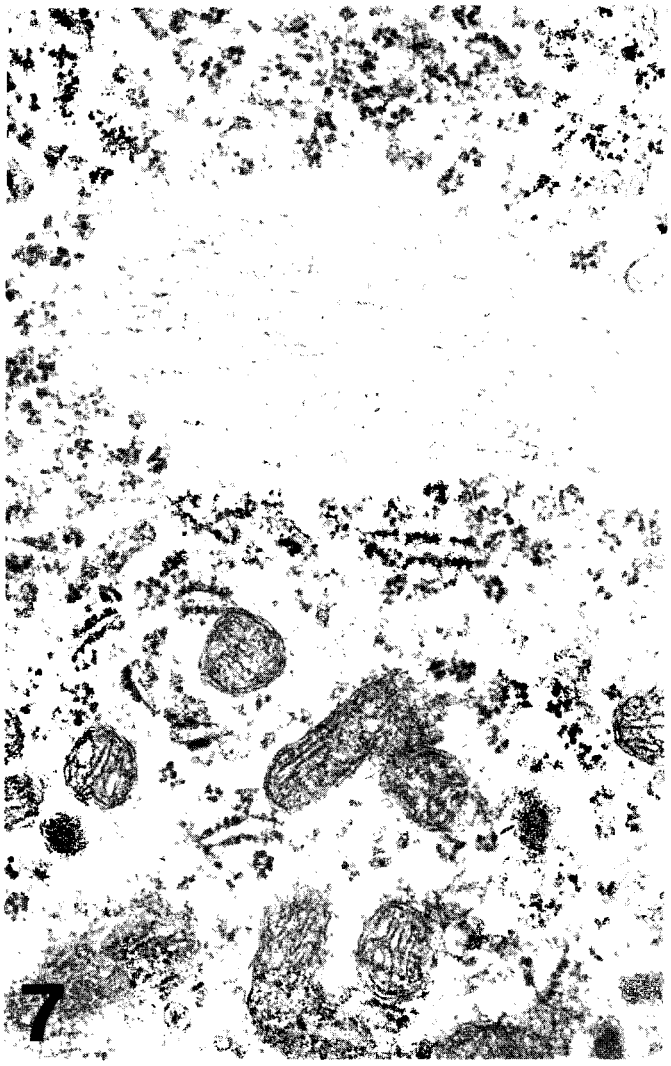
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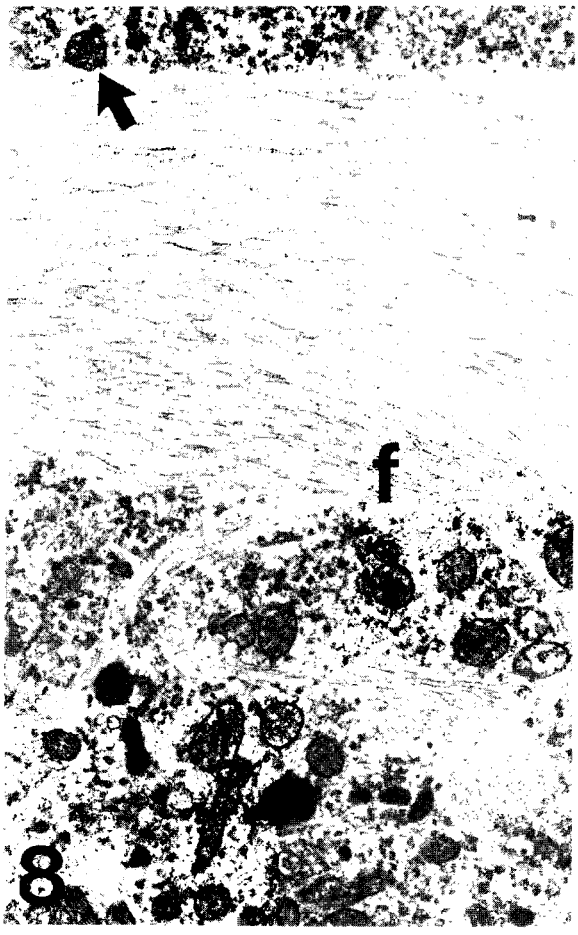
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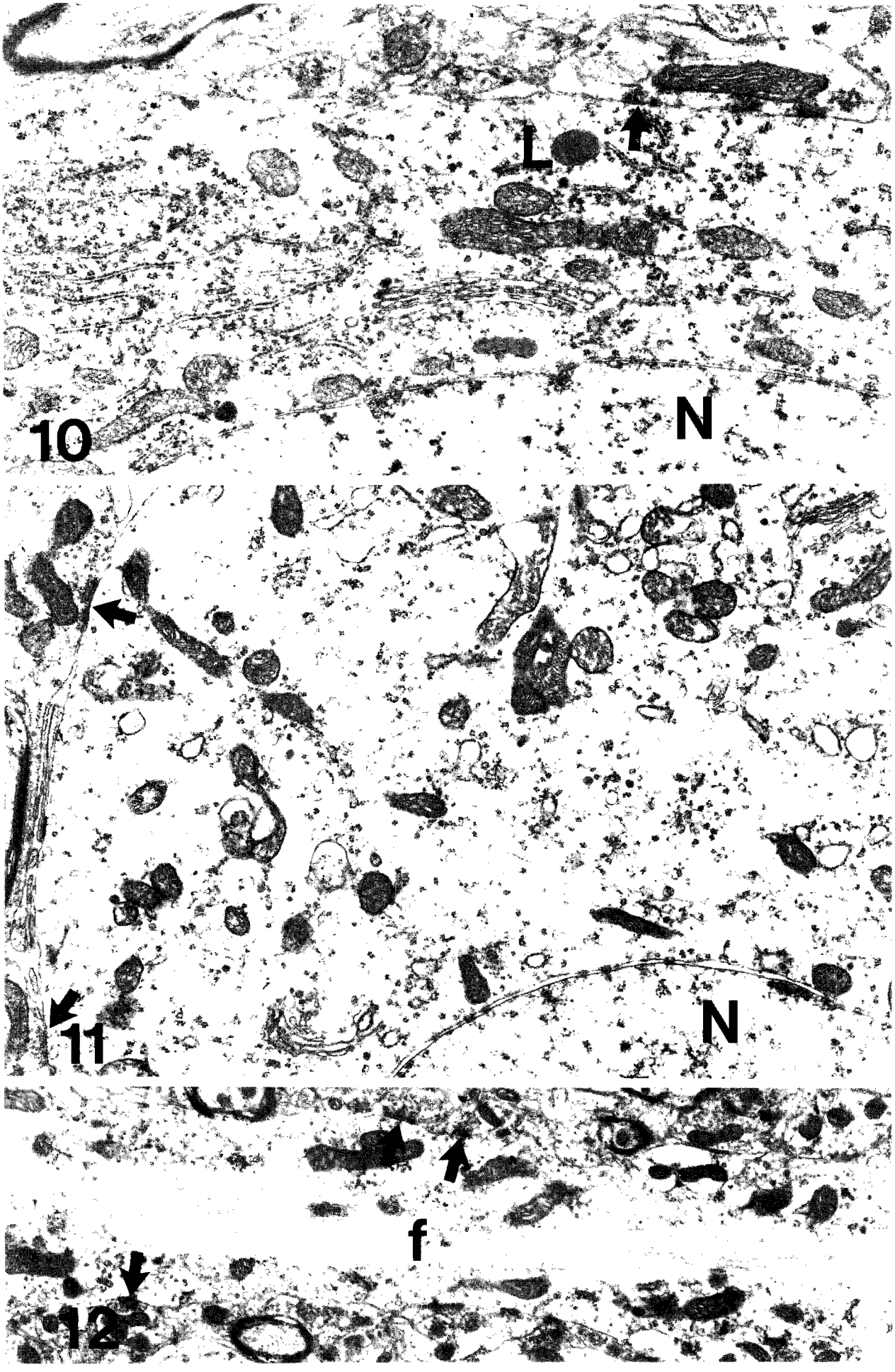
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Figs 4 and 5. Electron micrographs of a neuronal soma in the MTN from a colchicine treated preparation. **Fig. 4.** shows the nucleus (N) and a part of the perikaryal cytoplasm of this soma that contains cisternae of granular endoplasmic reticulum (R) adjacent to the nuclear envelope, and lysosomes and bundles of neurofilaments (f) in remaining regions of the soma. × 8,600. **Fig. 5** shows an enlargement of this soma with its nucleus (N), granular endoplasmic reticulum (R), lysosomes (L- indicates the one with an arrow in **Fig. 4**) and bundles of neurofilaments (small arrows). × 19,000

Figs. 6-9. Electron micrographs of other MTN somata from colchicine treated preparations. **Figs. 6** and **7** show a portion of a soma with its nucleus (N) and adjacent cisternae of granular endoplasmic reticulum (R). Note the bundle of neurofilaments (f) that displays crossbridges in **Fig. 7.** × 13,000 and × 45,000, respectively. **Figs. 8** and **9** show a larger bundle of neurofilaments (f). The mitochondria (arrow) is used for orientation between the two figures. × 11,000 and × 22,000, respectively

Fig. 10. Electron micrograph of a soma from the deep cerebellar nuclei of a non-treated preparation. The nucleus (N) and the perikaryal cytoplasm that displays lysosomes (L) appear normal. One terminal forms a symmetric axosomatic synapse (arrow). × 20,000

Fig. 11. Electron micrograph of a soma from the deep cerebellar nuclei of a colchicine treated preparation. The nucleus (N) appears normal but the perikaryal cytoplasm displays many clear areas that give it a watery appearance. Two terminals form symmetric axosomatic synapses (arrows). × 19,000

Fig. 12. Electron micrograph of a dendrite from the inferior colliculus of a colchicine treated preparation. This dendrite displays a bundle of neurofilaments (f) and axodendritic synapses (arrows). × 12,000

Discussion

The primary finding of this study is the differential accumulation of neurofilament bundles and lysosomes in somata of the MTN in colchicine treated preparations. In the MTN colchicine failed to produce a reduction in the somatic concentration of lysosomes. Instead, colchicine induced a massive accumulation of neurofilament bundles in the cytoplasm. In other neuronal populations examined colchicine led to a reduction in the concentration of somatic lysosomes, but did not produce an accumulation of neurofilament bundles in the cytoplasm.

The cytoplasm of MTN neurons often contained vast areas in which the normal complement of organelles was displaced by the neurofilament bundles. In these cells, a narrow rim of cytoplasm could be seen compressed against the nuclear envelope or the plasma membrane. In these areas of squeezed cytoplasm numerous organelles were observed.

We have previously shown that colchicine induces a redistribution of lysosomes and lipofuscin granules from the cell bodies of neurons to the dendrites (Gorenstein and Ribak, 1985; Gorenstein et al., 1985; 1988). A notable exception to this phenomenon was observed in the MTN. This finding was not unexpected since these neurons are pseudounipolar and have few or no dendrites (Walberg, 1984). It was not clear, however, from our results whether colchicine had any effect on the integrity of microtubules in MTN neurons. The results presented in this study indicate that in the MTN colchicine was effective in increasing the intrasomatic concentration of neurofilaments, presumably by disrupting their transport into axons. In addition, the unaltered concentration of lysosomes in the soma of these neurons confirms previous results showing that lysosomes do not enter axons following colchicine treatment.

Accumulations of large bundles of neurofilaments in the cytoplasm are characteristic of colchicine treated MTN neurons. Other investigators have also observed neurofilament accumulations following intervention with microtubule poisons. In rabbits and rat cells treated with small doses of colchicine (Peterson and Murray, 1966; Wisniewski and Terry, 1967; Wisniewski et al., 1968), neurofibrillary aggregates similar to those described in the MTN were observed in many neuronal populations. In contrast, we did not observe neurofilament bundles in the deep cerebellar and inferior colliculus neurons. Such an absence, however, may represent differences in the time course following colchicine injections or simply represent differences in the rate of neurofilament synthesis in various neuronal populations.

The finding of neurofilament bundles in the dendrites of the inferior colliculus of colchicine treated preparations is interesting. Typically, dendrites contain both microtubules and neurofilaments which are aligned parallel to each other (Peters et al., 1976). In the absence of microtubules, the neurofilaments in these dendrites form bundles similar in structure to those observed in the somata of MTN neurons. However, these dendritic bundles never achieve the size or proportion of those encountered in the MTN.

These observations are pertinent to some neurological disorders that display neurofilamentous bundles. For example, the neurofibrillary tangles found in the brains of Alzheimer's patients may reflect a disruption in the normal functioning of the microtubule system (Gajdusek, 1986; Gorenstein, 1987).

Acknowledgements. This work was supported by NIDA grant DA03131 to C.G. and NS18994 to C.G. and C.E.R. We gratefully acknowledge the technical assistance of Margot Brundage.

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Accepted November 12, 1987