COLCHICINE REDUCES MYELIN THICKNESS AND AXOPLASM VOLUME

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A Silastic cuff containing either colchicine (1% w/v) or no colchicine was placed around the lingual-chorda tympani nerve of the Mongolian gerbil. After 3 days of exposure to colchicine, the mean period of the myelin sheaths was 23% less than the period observed in nerves treated with cuffs lacking colchicine, while the average number of lamellae was unaltered. At the same time colchicine reduced the volume of axoplasm by an average of 19%, an effect which was independent of fiber diameter.

As part of a series of investigations on the neurotrophic maintenance of taste buds and taste responses [12, 13, 16], the present research was designed to evaluate the ultrastructural changes in the lingual-chorda tympani nerve caused by chronic exposure to colchicine. Earlier investigations had found that colchicine reduced the number of microtubules, and also increased the prominence of filaments in both neurons [2, 3, 5, 17] and glia [4–6, 11]. In addition, colchicine is known to impair both fast [2, 8, 15] and slow [5, 7] axonal transport. Here, we describe observations on colchicine-induced loss of axoplasm and thinning of myelin.

In order to correlate morphological changes in the nerve with physiological changes in taste activity, we evaluated the ultrastructural changes in axons exposed to colchicine for 3 days, by which time colchicine had impaired axonal transport and substantially reduced chorda tympani taste responses [16]. A nerve cuff of Silastic (Medical Adhesive Silicon Type A, Dow Corning) was implanted around the lingual-chorda tympani nerve in each of 12 Mongolian gerbils (*Meriones unguiculatus*, 5–9 months old, 56–74 g body weight) by methods described elsewhere [16]. Six colchicine cuffs (1% w/v) and six control cuffs lacking colchicine were used. Three days after cuff implantation, the gerbils were anesthetized with ketamine HCl (330 mg/kg body weight i.m.) and sodium pentobarbital (12.5 mg/kg

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i.p.). The unbranched, 5.5 mm segment of the lingual-chorda tympani nerve, 2 mm proximal and 2 mm distal to the cuff, was removed along with the corresponding segment of the normal contralateral nerve. The two segments were processed identically. Immediately after removal, the nerves were fixed for 2 h in 2% glutaraldehyde in 0.2 M sucrose buffered with 0.1 M cacodylate (pH 7.35), rinsed in the buffered sucrose, postfixed for 2 h in buffered 2% OsO_4 and rinsed again. The nerves were stained in 2% aqueous uranyl acetate for 1 h, dehydrated in ethanol, and embedded in Spurr's medium (Polysciences). Cross-sections were cut from the nerve segments just distal to the cuff and at a corresponding level in the contralateral nerves. Sections were stained with lead citrate and were examined in a Philips EM-300 electron microscope.

Analysis of electron micrographs was aided by a digitizing graphics tablet and microcomputer (Apple Computer, Inc.). All measurements were made without knowledge of the nerve treatment. Myelin thickness (distance between inner and outer lamellae) was determined by averaging measurements (typically 6-8) taken at 1 µm intervals around the perimeter of each axon's cross-sectional profile. The internal fiber diameter was computed from measurements of the circumference of the innermost myelin lamella. Axoplasm loss was quantified by measuring the cross-sectional area enclosed by the axolemma and expressing this area as a percentage of the area enclosed by the innermost myelin lamella.

From the examination of nerve ultrastructure, it was clear that the Schwann cells were affected by colchicine. In the colchicine treated nerves the Schwann cells had a more electron-dense cytoplasm and more prominent smooth endoplasmic reticulum with enlarged cisternae.

The ultrastructural appearance of axons treated with the control cuffs (Fig. 1A) was similar to the normal appearance of the untreated contralateral nerves. In contrast, in colchicine-exposed myelinated axons the number of microtubules was substantially decreased, the neurofilaments were more prominent and often longitudinally disoriented, and small clusters of mitochondria were frequently present in the axoplasm. Colchicine also reduced the volume of axoplasm, which led to the retraction of the axolemma away from the innermost lamella of the myelin (Fig. 1B). As the volume of axoplasm decreased, it was not uncommon for the sheaths to collapse inward, producing folds in the myelin. In six colchicine-treated nerves there was a mean 19% loss in axoplasm volume compared to six 3-day control cuffed nerves ($P<0.02$, t test, two tailed). The actual reduction in volume was probably greater than 19% because the concurrent infolding of the myelin effectively reduced the area enclosed by the myelin sheath. As calculated from the internal circumference of the sheath, the internal fiber diameters in control-cuffed nerves (2.43 ± 1.03 µm, M ± S.D.) were not significantly different from those of fibers exposed to colchicine (2.31 ± 0.99 µm, $P>0.1$), indicating that similar samples of axons were compared. We used internal, and not external, fiber diameters to avoid confounding measurements of axoplasm loss with the concurrent thinning of the
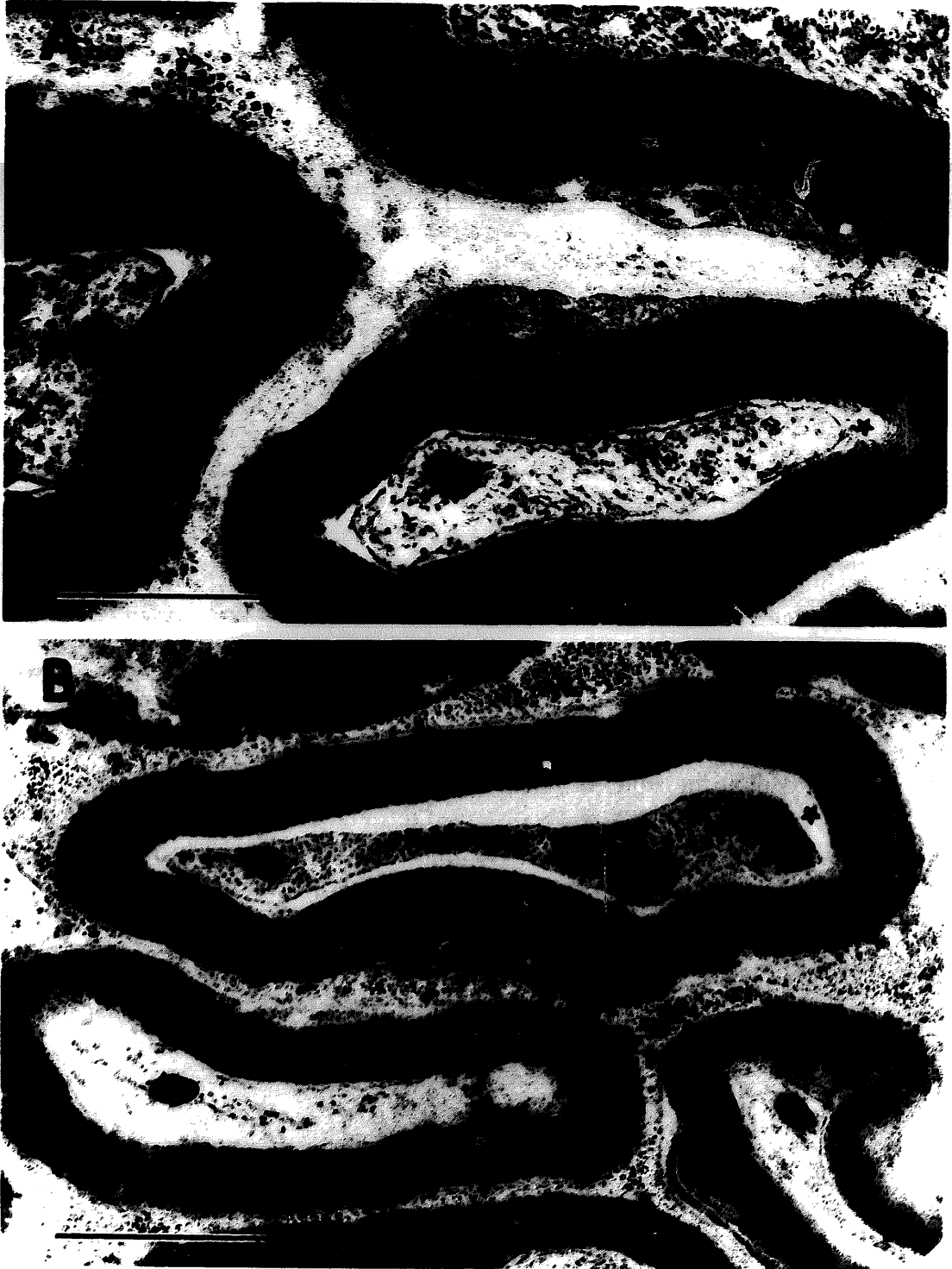
Fig. 1. A: with a control Silastic cuff lacking colchicine the appearance of the axons after 3 days is similar to normal uncuffed nerves. Note the abundant microtubules. The shape of the axon profiles is representative of those present. B: exposure to a 1% colchicine cuff for 3 days reduced the number of microtubules and the thickness of the myelin. Adjacent axons showed differing degrees of axoplasm loss. The starred axon (*) has 27 lamellae like the starred axon in A, but has thinner myelin. Bars = 1 μm.
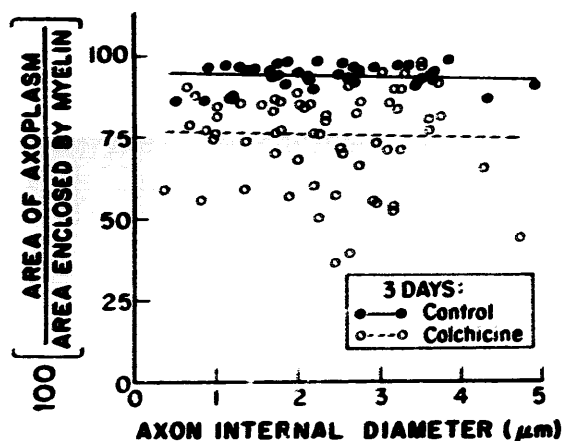
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Fig. 2. Colchicine produced a significant reduction independent of fiber size in the percentage of axonal cross-sectional area occupied by axoplasm. In nerves treated with control cuffs, as in normal nerves, the axoplasm nearly filled the area surrounded by myelin. Lines are the least squares best fit. n = 68 (colchicine) and 37 (control cuff).

myelin sheaths (see below). The near zero slope of the least squares best fit line in Fig. 2 (dashed line) indicates that the percentage of axoplasm volume lost was independent of fiber diameter, in contrast to an earlier report [5].

It is evident from the variability in Fig. 2 that axons reacted dissimilarly to the colchicine treatment. Axons with extensively reduced axoplasm were scattered throughout the nerve. When both the exposure to colchicine and fiber diameter were controlled by comparing adjacent fibers of similar diameter, substantial differences in axoplasm loss were still frequently observed (cf. Fig. 1B). It is reasonable to conclude that the variation among axons in axoplasm loss was primarily determined by differential susceptibility of axons to colchicine rather than by differences in diameter or colchicine exposure.
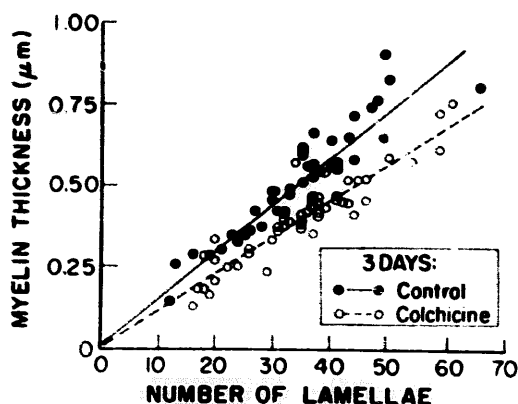

Fig. 3. For all fiber diameters the thickness of the myelin sheath was reduced by 3 days of exposure to colchicine. The slope of the line gives the period for both control and colchicine-treated groups. Lines are the least squares best fit. n = 50 (colchicine) and 46 (control cuff).

The ability of colchicine to impair axonal transport has been well documented [2, 8, 15] and was verified in the present research by observing impaired cholinesterase transport [16]. The accumulation of mitochondria provided additional evidence for impaired axonal transport. We hypothesize that the colchicine-containing cuff reduced axonal transport, which led to a local depletion of axoplasm.

Myelin thickness and the number of lamellae were measured in axons from six nerves treated with colchicine-cuffs and six nerves treated with control cuffs. Colchicine reduced the mean thickness of the myelin sheath to 0.40 ± 0.13 μm from the normal values found with control cuff treatment (0.51 ± 0.16 μm). As an example, the two starred axons (*) in Fig. 1 each have 27 lamellae, yet the myelin sheath of the colchicine treated axon is 32% thinner. The colchicine-induced thinning of the myelin sheaths was directly proportional to the number of lamellae. The Pearson correlation coefficients are $r = +0.92$ (control cuff) and $r = +0.93$ (colchicine-containing cuff) for the least squares best fit lines in Fig. 3.

The myelin sheath's period (distance between the centers of the major dense lines) was calculated in individual axons by dividing the myelin thickness by the number of lamellae. The mean period in axons with control cuffs was 14.7 ± 1.8 nm, which is similar to other electron microscopic measurements of myelin period in mammalian peripheral nerves [10, 14]. Three days of colchicine treatment decreased the mean period by 23% to 11.3 ± 1.7 nm. The reduced myelin period is graphically displayed by the slopes of the lines in Fig. 3 ($P<0.01$, t test). The thinning of the myelin was not the result of lost lamellae; colchicine treated axons had a mean of 35.2 ± 11.0 lamellae compared to 34.7 ± 10.5 lamellae for axons of nerves with control cuffs. Nor was the shrinkage confined to either the inner or outer lamellae; at 150,000X the reduction in period appear to be even across the width of the sheath.

The breakdown of myelin caused by other toxic chemicals generally results from direct attack on the myelin or from breakdown secondary to the destruction of the glial cells [9]. In both cases myelin breakdown is characterized by the formation of vacuoles at the intraperiod lines [1, 18]. However, colchicine treatment for 3 days was not associated with vacuole formation. Nonetheless, these more subtle effects of colchicine upon myelin may have stemmed from changes in the Schwann cells [9].

In summary, the chronic application of a colchicine-containing Silastic cuff to the lingual-chorda tympani nerve produced a partial loss of axoplasm and thinner myelin lamellae. Specifically, three days of colchicine treatment produced a mean 19% loss of axoplasm volume in both large and small myelinated fibers and a 23% reduction in the myelin period.

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