Axotomy Mimicked by Localized Colchicine Application

Abstract. *Comparable depression of synaptic transmission in the avian ciliary ganglion resulted from either section or localized colchicine treatment of the ciliary nerves. Both colchicine treatment and axotomy produced similar changes in RNA distribution in the cell bodies as well. Colchicine did not directly affect transmission, and action potential propagation along the ciliary nerves was normal. Interference with axoplasmic transport of material in both cases is postulated to signal the observed chromatolytic changes.*

Axotomy, or section of a nerve cell axon, induces anatomical as well as biochemical changes in the soma of neurons, especially those connected with the periphery (*1*). These changes will all be referred to under the general term of chromatolysis. In autonomic ganglia and spinal cord motoneurons axotomy has been shown to affect synaptic transmission onto the parent cell body as well (*2*). Various suggestions have been proposed as to the nature of the signal that initiates chromatolysis (*3*). In the present experiments an attempt was made to distinguish between some of the possible signals for chromatolysis. It is possible that substances carried by bidirectional axonal flow (*4*) could be involved in signaling the soma to initiate chromatolytic changes. Since colchicine has been shown to block at least the rapid components of axonal flow (*5, 6*), it was of interest to compare the effect of application of colchicine with the effect of axotomy.

Axotomy of a neuron results in the loss of axoplasm and the disruption of the Schwann cell sheath around the axon. The soma could be depolarized or otherwise affected by impulses from the cut end, and the axonal transport of any material from the periphery would be blocked as well. In contrast, the application of colchicine would not directly injure the axonal membrane, nor result in loss of axoplasm, and only substances carried by axonal transport would fail to reach the soma. The continuity between soma and periphery would not be physically interrupted, and slow axonal transport should continue to function at least partially (*5*). A recent study (*7*) has shown that interference with axonal flow by colchicine depressed synaptic transmission in the nerve endings of the treated cells, and it seemed worthwhile to see if chromatolytic changes could be brought about by similar axonal application of colchicine.

The experiments were performed in the chick ciliary ganglion, and chromatolysis was assessed electrophysiologically as modifications in synaptic transmission through the ganglion (*8, 9*) and as alterations of RNA distribution in ganglion cells. The avian ciliary ganglion is a useful model on which to study the effects of axotomy, because only one or two of the four to six ciliary nerves can be sectioned, leaving the intact branches to serve as controls (*8*).

Section of the postsynaptic ciliary nerves results in a progressive failure of transmission in the ciliary cells (*8*) of the avian ciliary ganglion, which can be quantified by the following procedure. A suction electrode was used to record the response to maximal preganglionic electrical stimulation from each postsynaptic (postganglionic) branch of the ciliary nerves. The nerve responses were amplified and photographed from an oscilloscope screen. The ganglion was subsequently removed and the postsynaptic nerve directly stimulated, the response being recorded from the same postsynaptic branches as before (Fig. 1, upper). By comparing the area under the response curves for pre- and postsynaptic stimulation, the degree of transmission through the ganglion can be estimated (Fig. 1, A and A') (*10*).

Axotomy or local application of colchicine was carried out in 1- to 2-day-old chicks, anesthetized with methoxyflurane. A small incision was made in the sclera of the eye, and the branches of the ciliary nerves were exposed. Several nerves were either sectioned with iridectomy scissors, or had colchicine applied to them in the following manner previously used by Dahlström (*11, 12*). Small pellets of cotton (< 1 mm in diameter) were soaked in a 10 to 20 percent aqueous solution of colchicine and allowed to dry. One of these pellets was placed in contact with the exposed nerves for 10 minutes and then withdrawn. The exposed area was thor-

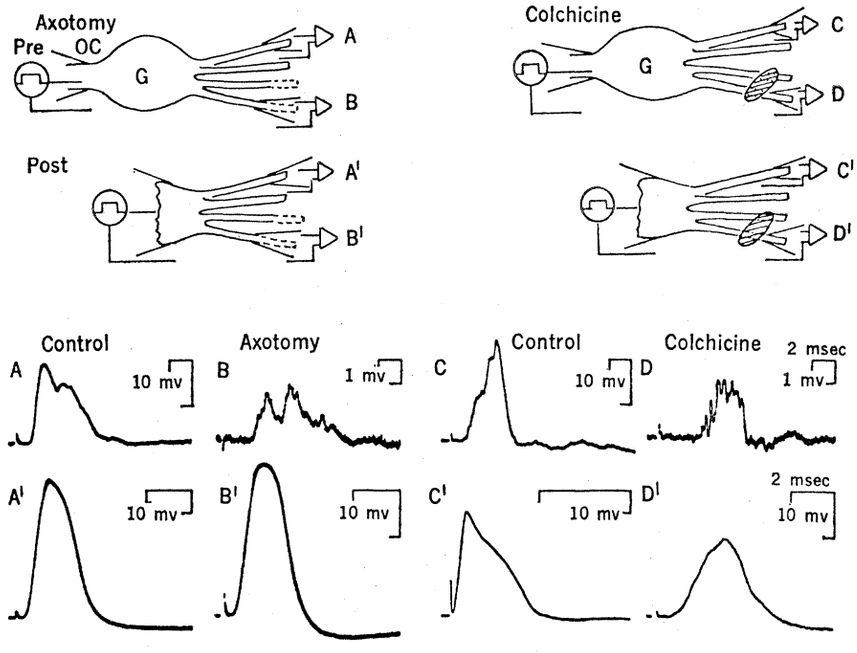

Fig. 1. Method for estimation of degree of synaptic transmission through the isolated ciliary ganglion (*G*). Suction electrodes were used to stimulate the presynaptic oculomotor nerve (*OC*) and to record from various postsynaptic branches. At top left, responses to presynaptic stimulation were recorded from an intact (A) and axotomized (B) postsynaptic nerve 3 days after axotomy. The ganglion was then removed and the postsynaptic nerve stimulated directly (A', B'). The responses obtained are seen at bottom. At top right, the same procedure was used to estimate degree of transmission in an intact (C) postsynaptic nerve and one that had been treated with a colchicine pellet 3 days earlier (D). The dashed oval indicates the site of colchicine application. Since recordings were always made distal to the site of colchicine application, conduction distance in these branches was usually longer than in control branches, resulting in longer latencies, as can be observed in D and D' at lower right.

oughly washed with Tyrode solution, which was removed with gentle suction. In control experiments, the nerves were treated in a similar way, but colchicine was omitted from the pellets. No changes were observed in these conditions. The wound was then sutured, and the birds recovered from the experimental procedure without complications. Ganglionic transmission was tested in isolated ganglia, superfused in a bath, at different intervals after the surgical procedure (from 10 hours to 10 days).

The typical effects of localized colchicine application (right) and axotomy (left) are compared in Fig. 1. On the left, the response to preganglionic electrical stimulation recorded from an intact (A) and 3-day axotomized branch (B) is shown. Postsynaptic stimulation (A′) produced a response of area comparable to that of the presynaptically evoked response (A) in the intact branch. However, in the axotomized branch the response to presynaptic stimulation (B) was only a small percentage (17 percent) of the response to postsynaptic stimulation (B′), indicating severely depressed ganglionic transmission.

Similar effects (Fig. 1, right) were observed when colchicine was applied locally. The postsynaptically recorded response from the intact branch was of the same area whether stimulation was pre- (C) or postsynaptic (C′), indicating 100 percent transmission. The proportion of transmitting cells in the colchicine-treated branches (D), however, was only 15 percent. Since the neighboring untreated branch was normal, it can be inferred that colchicine did not diffuse to the adjacent nerves or to the ganglion. Diffusion of the drug would depress transmission in all branches because it has been shown that colchicine directly affects transmitter secretion (*13*). This was not observed. Most control branches transmitted normally (110 percent ± 11, mean ± S.E., Fig. 2).

Another possibility is that colchicine at the concentrations used could directly injure the axon membrane, resulting in a virtual axotomy. This was ruled out by recording from the treated axons distal to the site of the colchicine application (Fig. 1, D and D′). It was thereby ascertained that in most cases colchicine application did not injure the axonal membrane, because impulses were conducted through the site of colchicine application [see also (*7, 14*)]. Nerves appeared macroscopically normal with no shrinkage, swelling, or other abnormal characteristics. The response recorded distal to the application point could also be compared with the proximally recorded response. In a few cases partial damage of the ciliary nerves was detected by this means and could result either from a direct toxic effect of the drug or from mechanical or osmotic effects during the operative procedure. However, even in these cases the transmission in the cell bodies of that portion of the nerve that was intact was still depressed to about the same level that would be expected from axotomy.

Furthermore, the conduction velocity of either untreated (3.8 ± 0.3 m/sec, mean ± S.E.) or colchicine-treated (3.7 ± 0.3 m/sec, mean ± S.E.) nerve branches in colchicine preparations did not differ from control values (3.5 ± 0.3 m/sec, mean ± S.E.). Likewise, axotomized branches conducted at 4.0 ± 0.2 m/sec (mean ± S.E.) and intact branches from the same preparations at 4.1 ± 0.2 m/sec (mean ± S.E.) (*15*). Thus, the localized application of colchicine did not mechanically disrupt axons or myelin sheathes, as impulses were conducted at normal velocity past the colchicine site.

Since the effects of axotomy and colchicine treatments were maximal from 2 to 7 days, the data from this time interval were pooled (Fig. 2). Although not shown, the time of onset of depression of transmission in the two cases was the same (20 to 24 hours). Transmission was depressed in axotomized ganglia to 19 percent ± 3 (mean ± S.E.) of control values, and in colchicine-treated ones to 29 percent ± 4 (mean ± S.E.). The slight difference may result from the fact that when the nerve is cut, all axons are obviously

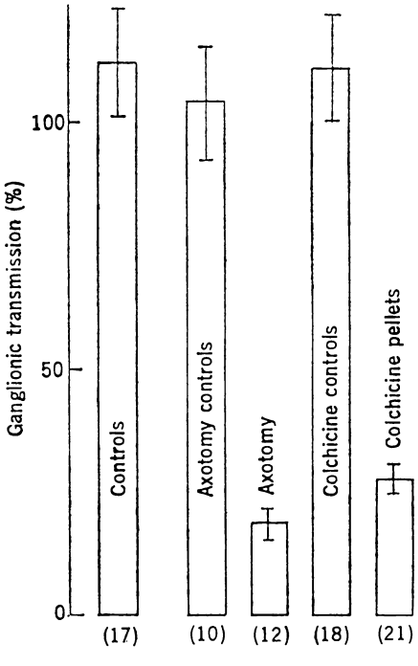

Fig. 2. Effect of axotomy and localized colchicine application on ganglionic transmission. Bars represent mean percentage of transmission through the ganglion in various control, axotomized, and colchicine-treated nerves. *Axotomy controls* and *Colchicine controls* refer to the untreated branches in each of the experimental ganglia, and did not differ from control animals. The numbers in parentheses indicate the number of observations and the standard error is represented by the vertical lines.

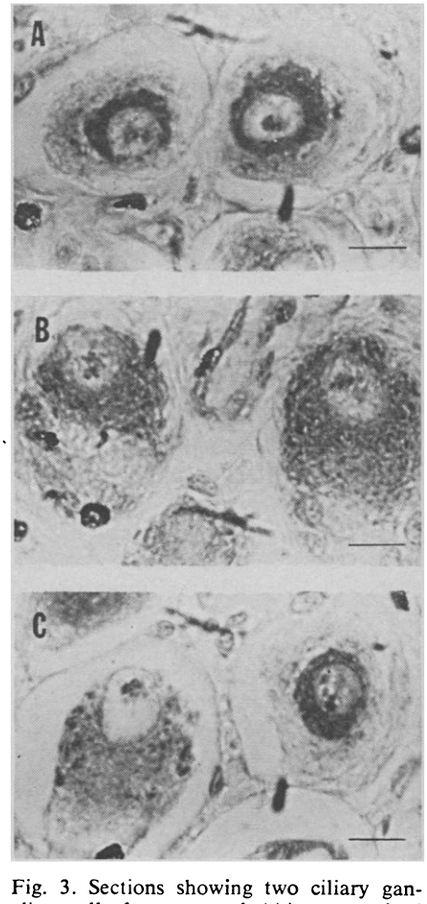

Fig. 3. Sections showing two ciliary ganglion cells from normal (A), axotomized (B), and colchicine-treated (C) ganglia stained with Pyronine–Malachite Green. The normal cells (A) show a distinct ring of perinuclear RNA. In contrast, the cytoplasm of the axotomized cells (B) is uniformly stained and lacks a perinuclear ring. Colchicine treatment (C) of only two of the five ciliary nerves produced some cells with normal appearance (right) and others with the typical chromatolytic staining pattern (left). Calibration bar represents 10 μm.

severed, while in some cases the applied colchicine may not have reached all axons in sufficient concentration. Therefore, colchicine results in transmission changes that are similar in both time course and magnitude to those produced by axotomy.

Another independent method was used to assess chromatolysis and is based on the well-documented changes in the distribution of Nissl substance in chromatolized cells (*1*). Change in RNA distribution, detected with Pyronine–Malachite Green, a stain specific for RNA (*16*), closely paralleled the modification on the distribution of Nissl substance previously described in these same ganglion cells (*17*). In one ganglion all of the ciliary nerves were sectioned, and in another ganglion two of the five ciliary nerves were treated with colchicine pellets. Three days later the ganglia were removed and together with a control ganglion stained with Pyronine–Malachite Green. Serial sections 8 μm thick were cut and all sections were observed with light microscopy.

All ciliary cells (*18*) in the control ganglion possessed a dense perinuclear ring, the remainder of the cytoplasm staining very faintly (Fig. 3A). In the axotomized ganglion the cytoplasm of all ciliary cells was uniformly stained and perinuclear rings were absent (Fig. 3B). In the colchicine-treated ganglion, approximately 40 percent of the ciliary cells (Fig. 3C, left) showed the typical chromatolytic staining pattern depicted in Fig. 3B. The remainder of the cells (Fig. 3C, right) appeared normal, with dense perinuclear rings.

The approximate proportion of ganglion cells showing the chromatolytic staining pattern in the colchicine-treated ganglion was expected, for although the number of axons in the colchicine-treated and nontreated branches was not counted, the treated branches, as judged from their size, composed roughly half of the ciliary nerve population.

In the present experiments chromatolysis was assessed by two widely different methods. In both distribution of RNA and depression of transmission, the effect of colchicine was comparable to that produced by axotomy. If axonal transport does carry the signal for chromatolysis, the fast component of this flow would be implicated, since chromatolytic changes were seen to occur within 1 day of axonal section [present experiments and (*16, 19*)]. Colchicine has been shown to block fast axonal transport (*5, 6*), and it is therefore tempting to speculate that some substance carried by fast axonal flow is indeed the signal for chromatolysis.

Several other hypothetical signals are excluded by the present results: injury of the axonal membrane, loss of axoplasm, electrical changes, and disruption of the myelin sheath. Another possibility, compatible with the present experiments, is that both axotomy and colchicine block the flow of some trophic substance supplied by the periphery. However, experiments of Watson (*20*) utilizing botulinum toxin have eliminated this hypothesis. The remaining alternative is that some substance circulating within the axon by axonal flow either causes or represses chromatolysis. In the former case some excitatory substance which might normally be consumed in the nerve ending would accumulate in higher concentration in the soma after axotomy or blockage of axonal flow. In the latter case, some inhibitory substance might be utilized by the growing portion of the neurite in, for example, membrane synthesis, thereby lowering its concentration in the soma and bringing about chromatolysis. Watson (*21*) favors the idea that axon sprouting is the common factor in chromatolytic RNA synthesis brought about by either axotomy, botulinum toxin, or collateral sprouting. However, colchicine has not been observed to cause nerve sprouting, and such sprouting would have to be rapid in order to explain the rapid onset of chromatolysis [(*16, 19*) and the present experiments].

While RNA synthesis is perhaps the primary sign of chromatolysis, the study of other effects of axotomy, such as depression of transmission and synthesis of enzymes, would be useful. For RNA synthesis may occur both after axotomy and with collateral sprouting, but the total response of the soma may well differ in these two cases. Comparison of the entire array of changes produced by axotomy, colchicine application, and botulinum toxin might distinguish between the multiple effects of axonal injury and contribute to the understanding of both the nature of chromatolysis and the signal causing it.

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