EFFECT OF COLCHICINE ON
NEURONAL EXCITABILITY

THESIS

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Fulfillment of the Requirements

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By

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The abundance of microtubules in receptive dendrites suggests they may function in sensory transduction. Responses of frog muscle spindle receptors and joint receptors is inhibited within 25 minutes by 50 mM colchicine, a microtubule-disrupting agent. The inhibition is reversible upon removal of colchicine, and the time course of recovery is comparable to that of inhibition. Frog olfactory responses are briefly inhibited by washing the olfactory mucosa with perfusion fluid. Colchicine accentuates the inhibition and substantially retards the rate of recovery in a dose-dependent fashion.

Colchicine does not affect axonal conduction, nor the oxygen uptake of isolated crab or frog leg nerves. The inhibitory action of colchicine is therefore an effect on the electrical excitability of the receptive dendrites or soma, and not an effect on axonal conduction.
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CHAPTER I

INTRODUCTION

Intracellular Microtubules

Microtubule (MT) is the collective name given a class of fibrillar protein found in perhaps all eukaryotic cells. Microtubules scattered throughout the cytoplasm exist in all protozoa, animal, and plant cells (Porter, 1966; Hepler and Palevitz, 1974), and in at least two species of bacteria (Iterson et al., 1967; Pope and Jurtshuk, 1967; Vela et al., 1970). The introduction of glutaraldehyde fixation for electron microscopy (Sabatini et al., 1963) revealed the wide distribution of MT. Osmium tetroxide, the fixative of choice prior to the mid 1960's, destroyed most MT in cells, leaving intact only the most stable MT, such as those found in cilia and flagella.

Structurally, MT are straight, cylindrical structures, 24 nm ± 2 nm in diameter, with a hollow-appearing (non-staining) 15-nm lumen. Electron micrographs of negatively-stained material taken at high magnification almost always show 13 protofilaments in the MT wall (Ambrose and Easty, 1975; Guttman, 1971; Olmsted and Borisy, 1973; Erickson, 1975), but from 11 to 15 protofilaments have been observed (Snyder and McIntosh, 1976). The protofilaments are assembled from the monomeric MT protein, tubulin (Soifer, 1975).
The appearance of MT in electron micrographs is astonishingly similar across phylogenetic lines. Chemical composition, immunological reactivity, and drug sensitivity are also virtually identical. They are probably more similar across phyla than any other cellular organelle, with the possible exception of the plasma membrane, which itself varies considerably among different tissues within an organism. With so much conserved structure, MT are certainly evolutionary homologs (Margulis, 1973).

Microtubules make up the typical 9 + 2 arrangement of motile cilia and flagella, as typified in sperm tails (Porter, 1966; Ringo, 1967; Manton, 1952; Afzelius, 1959; Allen, 1968). Centrioles and basal bodies are also characterized by a similarly distinctive arrangement of MT (Fulton, 1971). The major framework of the meiotic and mitotic spindle in all known cases is composed of MT (Nicklas, 1971; Luykx, 1970). Cytoplasmic MT which occur singly or in unattached bundles are generally referred to as "free microtubules."

In animal cells, MT are characteristically arranged in parallel in the cytoplasm along cellular extensions. The most striking instances are the growing neurites and axons, and dendrites of developing and adult neuronal cells (Wuerker and Kirkpatrick, 1972; Peters et al., 1970). They are also characteristic of cytoplasmic extensions of cells in tissue culture (Taylor, 1966), and may form complex patterns in the axopodia of various protozoa (Tilney, 1971). Microtubules are also prominent in developing systems where change in
cellular shape is occurring; for example, in lens development (Piatogirsky et al., 1972; Pearce and Zwaan, 1970), neuronal outgrowth (Lyser, 1968; Tennyson, 1970; Yamada et al., 1970), and gastrulation (Tilney and Gibbins, 1969). The trophic agent, nerve growth factor, may stimulate growth by promoting MT synthesis (Levi-Montacini, 1976).

Other Fibrous Proteins in Neurons

The subject of this study is neuronal MT, but besides MT, two other types of fibrous proteins occur in nerve cells. These are categorized as "neurofilaments"--8-10-nm filaments similar to the "intermediate filaments" or "tonofilaments" found in other cells, and "microfilaments"--1-2-nm contractile, actin-like threads which occur in most eukaryotic cells (Yamada et al., 1970; Wessells et al., 1971; Fine and Bray, 1971; Weurker and Kirkpatrick, 1972). A clear biochemical and pharmacological distinction has now been drawn between the three major neuronal fibrous proteins, and their functional differences are becoming clear. Most nerve cells have fibrous protein in all regions of the neurons (soma, axon and dendrites), but MT and neurofilaments are particularly abundant in lengthy neuronal processes.

Functions of Neuronal Fibrous Proteins

Both MT and neurofilaments seem to be agents of growth of cell processes and structural support in developed cells. Microtubules also play a role in the axoplasmic transport of
neurotransmitters, metabolites, proteins, lipids, and other cellular constituents from the cell body to distal areas, where synthetic capacity is lacking. Many types of sensory receptors have dendrites which are modified cilia (Burnside, 1975), and the dendrites of such receptors contain intracellular MT in 9 + 2 or 9 + 0 pattern. Free MT are also very common in sensory dendrites. The most remarkable elaboration of dendritic MT is found in arthropod mechanoreceptors (Moran et al., 1971). For example, attached to the dome of the cockroach campaniform mechanoreceptor is a dendrite which contains 350-1000 free MT. Admittedly, some receptors may have outer segments which are totally free of MT (e.g., microvillar photoreceptors in invertebrates), but most receptor dendrites have at least one MT.

Atema (1973, 1975) argued that the abundance of MT in receptive dendrites suggests they may function in sensory transduction, the conversion of stimulus energy into electrical activity. Moran and Varela (1971) suggested that in cockroach mechanoreceptors, an external force moving dendritic MT could result in transmission of energy to the cell body, which could then trigger neuronal transmission through the usual membrane electrical events.

Microtubule Biochemistry

Microtubules from a wide variety of sources consist of two electrophoretically separable proteins (alpha and beta
of identical molecular weight (55,000 daltons), but different amino acid compositions (Bryan, 1974). Electron microscopy has shown MT to consist of globular subunits arranged in a cylinder with 13 subunits or protofilaments (Erickson, 1974) held together by weak, hydrophobic interactions.

Although MT are very similar, Behnke and Forer (1967) proposed four subclasses of MT based on structural and chemical stability.

1. Cytoplasmic or free MT. They are most labile and are highly sensitive to colchicine and low temperatures (2-4°C).

2. The central pair of 9 + 2 complex of motile cilia and flagella. These are sensitive to high concentrations of colchicine and prolonged low temperature (0°C).

3. The peripheral doublets of the 9 + 2 complex fall into two groups. The "B tubules" are insensitive to colchicine and decreased temperature, but disrupted by pepsin and high temperature (50°C).

4. The "A tubules" of ciliar and flagellar doublets are the most stable. They are insensitive to colchicine and low temperature, and only slightly sensitive to high temperature.

Olmstead and Borisy (1973) recognize the four classes of Behnke and Forer, but suggest that degrees of stability are
probably due to "slight modifications of subunits or association of other materials with tubules, such as intermicrotubular links." Thus in the opinion of Olmsted and Borisy, there may be little genuine difference between the four classes of tubules. The extensive pattern of cross bridges between both ciliary and flagellar MT suggests that cross-binding confers stability, i.e., low turnover and resistance to disrupting agents.

Tubules are probably formed by the addition of monomers or dimers at one end of the MT. Polymerized MT are in a dynamic equilibrium with a soluble pool of monomeric and/or dimeric subunit tubulin (Inoue and Sato, 1967; Johnson and Borisy, 1974). Treatment with disruptive agents which bind to the soluble subunits and make them unavailable for repolymerization shifts the equilibrium toward the dissociated or soluble state. Stabilizing agents, by contrast, shift the equilibrium to the polymerized state.

Colchicine and Other Spindle Poisons

Many compounds are now known to disrupt MT without necessarily affecting cellular metabolism, i.e., colchicine, podophyllotoxin, griseofulvin, and the vinca alkaloids. A number of lines of evidence (Weisenberg et al., 1968) show that colchicine (the classic "spindle poison") binds to soluble tubulin monomers which have a single high affinity site for colchicine. With colchicine bound, tubulin is
unavailable to recombine with formed MT, thus shifting the dynamic equilibrium toward the dissociated state (Wilson et al., 1973). Not all MT systems are susceptible to colchicine (e.g., ciliary MT). However, if a cilium or flagellum is amputated, it does not regrow in the presence of colchicine (Rosenbaum et al., 1969). Polymerized tubulin (in MT) is insensitive to colchicine, except possibly in very high concentration. The colchicine binding site is inaccessible in the assembled MT (Wilson and Maze, 1973). Other compounds with colchicine-like effects, such as podophyllotoxin and griseofulvin, compete for the colchicine-binding site. It is thus likely that these compounds have an identical mechanism of action (Wilson, 1975; Margulis, 1973).

The vinca alkaloids, vinblastine and vincristine, also bind to soluble tubulin, but unlike colchicine, the vinca alkaloid–tubulin complex reaggregates to form complex crystals. Vinblastine binds to tubulin at a different site than colchicine. Two types of binding sites exist: a high affinity binding site, which displaces guanosine triphosphate (GTP), and a low affinity site, characterized by the displacement of calcium when vinblastine attaches (Olmsted and Borisy, 1973).

Stabilizing Agents

Deuterium oxide (D₂O) displaces ordered water on polymerized tubulin molecules and stabilizes by shifting the dynamic equilibrium toward the polymerized state (Inoue and
Sato, 1967). Deuterium oxide can apparently "overstabilize" MT, because MT function is often disrupted (Salmon, 1975). This observation indicates that the dynamic equilibrium is essential for proper MT function. Another agent, dimethyl sulfoxide (DMSO) also has been reported to stabilize MT (Forer and Zimmerman, 1975), but further biochemical studies need to be done for both D$_2$O and DMSO, in order to determine their exact effect of tubulin and MT.

**Purpose of Study**

The present study investigates the possible inhibition of neuronal electrical conduction and sensory transduction by colchicine. Since MT are found in large numbers in axons and dendrites, they may play a role in excitation (Moran and Varela, 1972; Atema, 1973, 1975). Previous work by Schafer and Reagan (in press), has shown that colchicine reversibly inhibits electrical activity in arthropod mechanoreceptors.
CHAPTER II

MATERIALS AND METHODS

Animals

Horseshoe crab (Limulus polyphemus) desheathed leg nerves were used for initial excitability studies and respiration experiments (Wyse, 1967). Adult animals (18-24 cm maximum width of cephalothorax) were obtained from Gulf Specimen Co., Panacea, Florida, and kept in seawater of specific gravity of 1.021-1.023 and a temperature of 17-18°C. Seawater was prepared from Instant Ocean Synthetic Seawater mix obtained from Aquarium Systems, Inc., Eastlake, Ohio. All animals were used within four months of their arrival.

Grass frogs (Rana Pipiens), obtained from Carolina Biological Supply Co., Burlington, North Carolina were used for quantitative studies. Frogs of 4½-5 inches were kept in running tap water at a temperature of 18-22°C.

Electrophysiology

Axonal Conduction

Compound action potentials were recorded from horseshoe crab leg nerves (Wyse, 1967) and from the classic frog sciatic nerve preparation (Oakley and Schafer, 1978). Three nerves were used during each run of the experiment. Each frog nerve was placed immediately after isolation in amphibian perfusion
solution (Oakley and Schafer, 1978), and crab nerves were placed in seawater at room temperature (23°-26°C), where they remained for about fifteen minutes before recording. For recording, nerves were placed in a nerve chamber (Oakley and Schafer, 1978) on silver wire electrodes. A Grass Model S44 electronic stimulator and S1U5 isolation unit were used to stimulate each nerve at its proximal end. The compound action potential was recorded by a Grass P15 AC-coupled preamplifier, displayed on a Tektronix R5103N storage oscilloscope, and photographed when necessary, with a Tektronix C-5A oscilloscope camera. Conduction velocity was determined by recording the compound action potential at two points.

Three other measures of excitability were taken. The amplitude of the response (voltage) was measured, a strength duration curve established, and the chronaxie computed. Absolute refractory period was measured using twin pulses delivered by a Grass S88 stimulator used in conjunction with the Grass S44.

After the initial measurements were taken, nerves were placed in separate 10 ml beakers containing 10 mM colchicine and perfusion fluid or perfusion fluid only. These beakers were then incubated for fifteen hours at 17°C, long enough for colchicine to disrupt all neuronal MT. All measurements were repeated after incubation.
Joint Receptors and Muscle Spindles

Two isolated hind legs of a frog were used in each experiment, using the technique of Oakley and Schafer (1978). The peroneal nerve was exposed and lifted onto a fine silver wire electrode. Nerve impulses were amplified by a Grass P15 AC-coupled preamplifier, and displayed on a Tektronix R5103N oscilloscope. A Heathkit AA-18 audio amplifier and a loudspeaker were also used to audio-monitor responses. Both legs were pinned on wax in two separate petri dishes filled with amphibian perfusion solution. After about ten minutes during which the viability of each preparation was verified, the fluid in one of the petri dishes was replaced with 50 mM colchicine in amphibian perfusion fluid to disrupt MT in the preparation.

The muscle, extensor brevis profundus digiti III, was stretched by gently pulling its tendon or directly touching the muscle with a glass rod. Recordings were taken from both the control leg and the colchicine-treated leg at regular time intervals. Both audio and visual responses were noted, and these responses scored on a scale of one to four, with four representing the maximum response and one a minimum discernable response. A zero rating indicated no response. The colchicine-treated leg was rinsed with amphibian perfusion fluid in an attempt to reverse effects produced by colchicine. Experiments usually ran for about two hours and were all done at ambient room temperature, 23°C-26°C.
Olfaction

Electro-olfactograms (EOG's) were recorded from the olfactory mucosa of frogs anesthetized with tubucurarine (0.013 mg/10g body weight) injected in the dorsal lymph sac. After the olfactory mucosa were exposed by surgery, an active, fluid-filled capillary electrode was carefully lowered onto the surface of the mucosa, while an identical reference electrode was placed on exposed skull at the margin of the surgical opening. Active electrode position on the mucosa was adjusted until the best EOG was observed. Each amphibian perfusion fluid-filled micropipette was broken off and fire-polished to produce a tip diameter of about 40 μm. Each micropipette was held in a WPl-electrode holder, mounted on a Narashighe MM-3 micromanipulator.

The stimulating apparatus was similar to that described by Schafer (1977). Stimulation was programmed with a Grass S88 electronic stimulator, driving a low current relay (Potter Brumfield No. JR-1051), which in turn operated a 3-way electric valve (Skinner Valve Co., New Britian, Ct.) in the stimulating air line. Stimulating air from the building air system passed through activated charcoal at 1750 ml per minute into the stimulating system. Stimuli were of 0.5 sec. duration, given at 100-second intervals, with a control (blank) stimulation given 50 seconds after each active stimulus.
Amyl acetate was used as a standard odorant. A 2 x 2-cm filter paper containing 20 µl of purified amyl acetate was placed in a glass stimulating cartridge. Stimulating cartridges were prepared in a separate room, about 15 to 20 minutes before each experiment, and kept in tightly capped individual vials. A control (blank) cartridge contained untreated filter paper. The stimulating cartridge containing an odorant consisted of a glass tube 0.75 cm I.D. x 3.8 cm long, with a 7/15 ground glass fitting for attachment to the stimulating air line. A 2 x 2-cm filter paper containing the stimulatory odor was rolled and inserted to about the constriction next to the ground glass joint. The cartridge was placed in a closed system along the air line, and the odor was expelled when the valves opened. The orifices of both the active and blank cartridges were placed side by side 3 cm from the olfactory mucosa and at a 90° angle from it. An evacuation system continuously removed the odorant from the site and exhausted it to the outside of the building. A Faraday cage with solid walls shielded the preparation from electrical noise and room air currents, and prevented stimulatory odors from escaping into the laboratory.

The EOG was recorded by a Grass P16 DC-coupled preamplifier fed into a Tektronix AM502 differential preamplifier, and displayed on a Taktronix R5103N storage oscilloscope and recorded on paper with a pen recorder (Houston, Omni Scriber). For EOG recordings, band pass filters were set at the
following -3 dB points: 0.1 Hz (low frequency cutoff) and 0.1 KHz (high frequency cutoff). The amplitude of EOG's recorded with the lower frequency cutoff at 0.1 Hz did not differ from test recordings done in full DC mode.

Drops of increasing concentrations of colchicine in amphibian perfusion fluid were placed on the mucosal surface to disrupt the MT (Graziadel, 1971). The initial effect and recovery rate from washing were noted at each concentration. Equivalent concentrations of sucrose were used as controls.

Oxygen Uptake

Horseshoe crab legs were cut at the proximal end of the trochanter, and the leg nerves desheathed by severing the exoskeleton and muscles at the patello-tibial joint and slowly pulling the two segments apart (Wyse, 1967). Usually, several legs from the same animal were used for each experiment. Long and short nerve sections were combined in each Gilson respirometer flask to give a combined total nerve length of 10 cm. Controls were incubated in seawater, and experimentals in 10 mM colchicine in seawater. A solution of 1 mM glucose was added to all flasks as an energy source for the nerves. The bath temperature was maintained at 17°C. Oxygen consumption was measured every two hours for 10 to 12 hours. Carbon dioxide evolved during respiration was absorbed with freshly prepared 2.0 M potassium hydroxide.
CHAPTER III

RESULTS

Electrophysiology

Axonal Conduction

Representative action potentials produced by colchicine-treated and control (untreated) horseshoe crab leg nerves are shown in Figures 1 and 2. Incubation of crab leg nerves in 10 mM colchicine for 15 hours at 17°C did not block axonal conduction.

Quantitative measurements of axonal conduction were done with frog sciatic leg nerves (Figures 3-4). Incubation in 10 mM colchicine for 15 hours at 17°C increased the magnitude (voltage) of the action potentials produced by both treated and untreated nerves, an increase of 34-35% (Table 1). Treated and untreated nerves, however, showed an average reduction of 27-28% in conduction velocity. The absolute refractory periods of both groups increased, and both treated and untreated nerves became more excitable, as shown by the reduction in their chronaxie values. The effects of 10 mM colchicine treatment and control incubations on axonal conduction were not significantly different. Thus removal from the animal and incubation of frog nerves has several measurable effects on axonal conduction, but colchicine does not accentuate or otherwise alter these effects.
TABLE 1

Excitability of frog sciatic nerves measured before and after 15 hours of incubation at 17°C in either 10 mM colchicine in amphibian perfusion solution or perfusion solution only. The table represents the average values of 15 experiments. There is no statistically significant difference between control and colchicine treatments for any of the measured values.

A. Control (perfusion fluid only)  \[ N = 15 \]

<table>
<thead>
<tr>
<th>Conduction Velocity (m/sec)</th>
<th>Absolute Refractory Period (msec)</th>
<th>Chronaxie (msec)</th>
<th>Magnitude of Response to Supramaximal Stimulus (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Value</td>
<td>25</td>
<td>18</td>
<td>1.5</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>±4</td>
<td>±12</td>
<td>±0.14</td>
</tr>
<tr>
<td>% Change</td>
<td>-28%</td>
<td>+40%</td>
<td>-24.4%</td>
</tr>
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B. Treatment with 10 mM colchicine  \[ N = 15 \]

| Value | 26.1 | 19 | 2.13 | 2.93 | 0.0177 | 0.0127 | 1.45 | 1.90 |
| Std. Dev. | ±6.7 | ±5.56 | ±0.15 | ±0.25 | ±0.0037 | ±0.0057 | ±0.47 | ±0.56 |
| % Change | -27% | +37% | -28% | +34% |
**Joint Receptor and Muscle Spindles**

A high colchicine concentration (50 mM) was used in this experiment to compensate for the thickness of leg muscle through which the drug would have to diffuse. A spike counter or signal averaging device (integrator) was not available, so instead, visual and audio responses were used to estimate the magnitude and score responses. Figure 5 shows that colchicine inhibited responses 25 minutes after treatment began. Maximum inhibition was achieved by 55 minutes, but the responses were never completely abolished. The preparation recovered when washed in normal amphibian perfusion solution, reaching maximum recovery after 20 minutes of washing. Responses after recovery were never as strong as the initial responses before colchicine treatment. Treatment of leg muscles with 50 mM colchicine in situ with the circulation intact did not inhibit muscle spindle or joint receptor responses. Presumably the colchicine was swept away by the circulatory system.

**Olfaction**

Washing the olfactory mucosa of a frog reduced the magnitude of the electroolfactogram (EOG). As the mucosa dried, the EOG magnitude increased—presumably because odorants did not have to diffuse through as thick a water layer on the mucosal surface. Typically, the response magnitude was reduced to 55% of its initial value when
measured 50 seconds after washing. The addition of colchicine to the perfusion fluid resulted in a 90% decrease in response at 50 seconds, regardless of the colchicine concentration. Note that different concentrations of colchicine did not vary the initial decline significantly, but that recovery rate did show concentration dependency (Figure 6). A control wash with 20 mM sucrose in perfusion fluid recovered in 11.5 minutes, not measurably different from the recovery time with perfusion fluid alone. Increasing colchicine concentrations (1, 5, 10, and 20 mM) progressively retarded recovery. For example, the recovery period with 15 mM colchicine took 64 minutes. At 20 mM, recovery was never quite complete.

Oxygen Uptake in Horseshoe Crab Leg Nerves

The data collected (Figure 7) in these experiments show that the inhibitory effect of electrical activity by colchicine is not due to blockage of nerve metabolism. In a period of 10 hours, crab nerves incubated in 10 mM colchicine in seawater consumed an average of 455 ul O₂/hr per centimeter of nerve length, while nerves incubated in seawater only, for 10 hours, consumed 425 ul O₂/hr per centimeter of nerve length. Under the conditions of this study, 10 mM colchicine had no statistically demonstrable effect on the oxygen uptake by these nerves.
Figure 1. Compound action potential of a horseshoe crab leg nerve before and after extended treatment with seawater.

A) After soaking in seawater for 15 minutes at room temperature.

B) After incubation in seawater for 15 hours at 17°C. The nerve continued to respond. A decreased conduction velocity has resulted in increased separation of the unit action potentials which comprise the compound action potential.

Gain = 0.5 mV/div; Sweep rate = 1 m sec/div
FIGURE 1A. Before incubation in seawater

FIGURE 1B. After 15 hours incubation in seawater at 17°C.
Figure 2. Compound action potential of a horseshoe crab leg nerve before and after extended treatment with 10 mM colchicine in seawater.

A) After soaking in seawater for 15 minutes at room temperature.

B) After incubation in 10 mM colchicine and seawater for 15 hours at 17°C. The nerve continued to respond. Increased excitability, like that observed with frog leg nerves, probably accounts for the decrease in latency between stimulus and response in A and B.

Gain = 0.5 mV/div; Sweep rate = 1m sec/div
FIGURE 2A. Before incubation in 10 mM colchicine

FIGURE 2B. After 15 hours incubation in 10 mM colchicine at 17°C
Figure 3. Compound action potential of a frog sciatic nerve before and after extended treatment with amphibian perfusion solution.

A) After soaking in perfusion solution for 15 minutes at room temperature.

B) After incubation in perfusion solution for 15 hours at 17°C. The nerve continues to respond. Increased excitability has decreased the latency between stimulus and response in A and B.

Gain = 0.5mV/div; Sweep rate = 1m sec/div
FIGURE 3A. Before incubation in Perfusion fluid

FIGURE 3B. After 15 hours incubation in Perfusion fluid at 17°C
Figure 4. Compound action potential of a frog sciatic nerve before and after extended treatment with 10 mM colchicine in amphibian perfusion solution.

A) After soaking in perfusion solution for 15 minutes at room temperature.

B) After incubation in 10 mM colchicine and perfusion solution for 15 hours at 17°C. The nerve continues to respond.

Gain = 0.5 mV/div; Sweep rate = 1m sec/div
FIGURE 4A. Before incubation in 10 mM colchicine

FIGURE 4B. After 15 hours incubation in 10 mM colchicine at 17°C
Figure 5. Effect of 50 mM colchicine on joint receptors muscle spindles in frog legs. Responses were scored by the experimenter by listening to the response on a loudspeaker and visually estimating the density of action potential on the oscilloscope display during a single stimulus. A score of four represents the maximum response, and a score of one, a minimum discernable response. Zero would indicate no response (a condition never observed). Open circles represent the control frog leg soaking in frog perfusion solution and 50 mM sucrose and closed circles represent the experimental leg soaking in 50 mM colchicine in frog perfusion solution. The cross-hatched bar at the top of the graph indicates soaking in 50 mM colchicine in amphibian perfusion solution and the shaded bar indicates soaking in amphibian perfusion solution and 50 mM sucrose. This is a single experiment which is representative of 20 experiments. All other experiments showed comparable results with time variations of plus or minus five minutes common.
CONTROL: SUCROSE - PERFUSION (50 mM)  

FIGURE 5

TIME BASE (min)

RESPONSE LEVEL

COLCHICINE - PERFUSION (50 mM)
Figure 6. Recovery of electroolfactogram (EOG) amplitude after washing the olfactory mucosa with amphibian perfusion solution containing 20 mM sucrose as control and increasing concentrations of colchicine (1, 5, 10, 15 and 20 mM). Stimulus was amyl acetate air pulse at 100-second intervals.
Figure 7. Effect of 10 mM colchicine on oxygen uptake at 17°C by desheathed horseshoe crab (Limulus polyphemus) leg nerve. The averaged results of 15 trials are shown with two nerves in each flask. Open circles represent incubation in seawater plus 1.0 mM glucose. Closed circles represent incubation in seawater plus 1.0 mM glucose containing 10 mM colchicine. Incubation in 10 mM colchicine has no effect on O2 consumption under the conditions of this experiment. Error bars indicate standard deviation.
CHAPTER IV

DISCUSSION

Inhibition of Sensory Receptors by Colchicine

The observation of microtubules in a wide variety of sensory dendrites has led many investigators to believe that they might play a role in the initiation of electrical responses in receptors (Atema, 1973, 1975; Hejnowiez, 1972; Callatay, 1969; Palay et al., 1968; Sondelin, 1968). Moran and Varela (1971) tested this hypothesis by inhibiting mechanosensory responses in the cockroach leg using colchicine. Schafer and Reagan (1978) extended Moran and Varela's work, showing that the inhibition was reversible and dose-dependent. In the present study, frog joint receptors, muscle spindles, and olfactory receptors were also found to be colchicine-sensitive. Thus receptors from a variety of sources can be reversibly inhibited by colchicine. This action correlates with the known biochemical action of colchicine, whose binding to microtubule protein is reversible. Colchicine inhibition suggests that microtubules or microtubule protein are a part of the excitatory mechanism.

High concentrations of colchicine (1-50 mM) are required to produce inhibition in all reported experiments, including those described here. This, however, may be explained by
the fact that in each case, except the olfactory receptors, perfusion fluid must penetrate several layers to reach the neuron. In fact, it was the olfactory receptors where the lowest concentration tried (1 mM) was effective. If the action is intracellular, colchicine must penetrate the cells in sufficient quantity to produce an electrical effect which is evident within minutes. Cell biologists and biochemists have used lower concentrations of colchicine for experiments on cell growth and metabolism, but the time scale of their experiments has typically been in terms of hours or days. This investigation involves electrical activity, not metabolism or growth, and the time scale is in seconds and minutes.

Nerve Conduction

This study, like previous studies (Hinkley and Green, 1971; Rodriguez-Enchandia, et al., 1968) indicates that colchicine has little effect on action potential propagation. This supports the idea that colchicine concentrations sufficient to cause total microtubular disruption within axons do not affect axonal membrane properties or neuronal metabolism sufficiently to interfere with impulse conduction. The site of action of colchicine is probably the receptive dendrite or cell body, not the axon.
Possible Role of Microtubules in Sensory Transduction

The sensory process, dendrites, cell body, and axon all contain microtubules subject to disassembly by colchicine; hence microtubular disassembly is likely at all levels within bipolar sensory cells. Many investigators have suggested that microtubules are both skeletal and contractile in function (Porter, 1966; Tilney and Porter, 1965). The skeletal and/or contractile properties of microtubules, when disrupted by colchicine, could change the shape of the receptive dendrites or cell body. Thus the configuration of the plasma membrane in these regions, and consequently its permeability, could result in excitability changes. Significantly, only Moran and Varela (1971) have reported complete inhibition. The implication is that microtubules regulate membrane excitability, and are not direct generators of electrical responses. That is a function of the membrane.

Other investigators (Camerina and Bryant, 1976) found that epineural injection of colchicine would increase membrane resistance and decrease chloride conductance in rat muscles without an apparent block of nerve function. Ochs (1974) suggested that colchicine could inhibit electrical activity by disrupting the supply of materials transported by microtubular systems which are essential for sensory transduction. This mechanism and control of ionic
conductances or the nature of other substances involved would be a useful area of further investigation.

SUMMARY

Conclusions derived from this investigation may be summarized as follows: (1) The inability of colchicine to alter axonal conduction in comparison to controls suggests that axonal microtubules do not play a significant role in axonal excitability; (2) Colchicine and disruption of axonal microtubules do not disturb the oxygen uptake mechanism of the nerve; (3) The decline in response of receptor neurons during perfusion with colchicine suggests that microtubule protein may be involved in the excitatory process, probably at the dendrite; (4) Since none of these experiments completely inhibited sensory responses, the role of microtubules is probably supportive or regulatory, not primary.
REFERENCES


