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Editorial Views

Neuronal Microtubular Systems

THE ULTRASTRUCTURAL RICHNESS of cytoplasmic organization is emerging with improved optical and biochemical methodology. As a consequence, the division of labor among various intracellular structures and their interdependency have been revealed. It is now recognized that one of the most abundant and widely distributed intracellular structures is the "microtubule."[1]

Neurons possess organelles typically present in other types of cells, such as the mitochondria, ribosomes, lysosomes, and nucleus, and some that are unique, such as synaptic vesicles. In recent years it has become clear that the organelle most numerous in neurons is the microtubule.[2] Microtubules (MT) are characterized by their tubular appearance and are remarkably similar in the various cell types in which they occur. The overall diameter of a "tube" is approximately 250 A, and the wall surrounding the lumen is about 60 A thick (fig. 1). The tube length usually cannot be determined, but often it extends for several microns or more. Filamentous structures stream from surfaces of microtubules (fig. 2), and there is evidence that the ensheathing coat of axonal MT is of chemically different composition.[3-5] Indeed, in cross-sectional view, the ensheating materials appear to be part of a larger, gridlike system (resembling a spider web), with MT located at the bonding points.[6]

Microtubules in non-neuronal cells are often clustered in striking patterns, with distinct strands of material linking them together and holding them in fixed positions relative to one another. Thus, groups of microtubules assembled into specific patterns form a larger, multimicrotubular system within which the actions of the individual microtubules may be coordinated. These multi-microtubule assemblies are best known in protozoa (see ref. 6 for example) and in the so-called "nine doublets" in cilia, sperm tails, and flagella. Although the tubules running lengthwise in axons appear as single entities by traditional procedures of electron microscopy, recent studies using modified methods reveal strands between the axonal MT which may integrate the individual behavior of the MT.

Microtubules are a major component of the mitotic spindle in dividing cells and are prominent in elongated cellular processes. Direct counts of the numbers of microtubules per unit area have been made in a few cases[7]; they are more numerous in nonmyelinated than in myelinated axons, and they are more abundant in the axons in immature animals than in mature animals.[8] An extensive survey of MT in rabbit vagus-nerve nonmyelinated axons and in crayfish ventral-cord axons revealed considerable numerical variation among axons, with an average of 40 MT/sq μ of axoplasm.[9]

Microtubules tend to be unstable, that is, they readily break down into their composite molecules. There is considerable variation in the stability of microtubules from one cell type to another and even in different portions of the same cell. There are also differences in the lability of microtubules in response to drugs, including some anesthetics (ref. 9; also

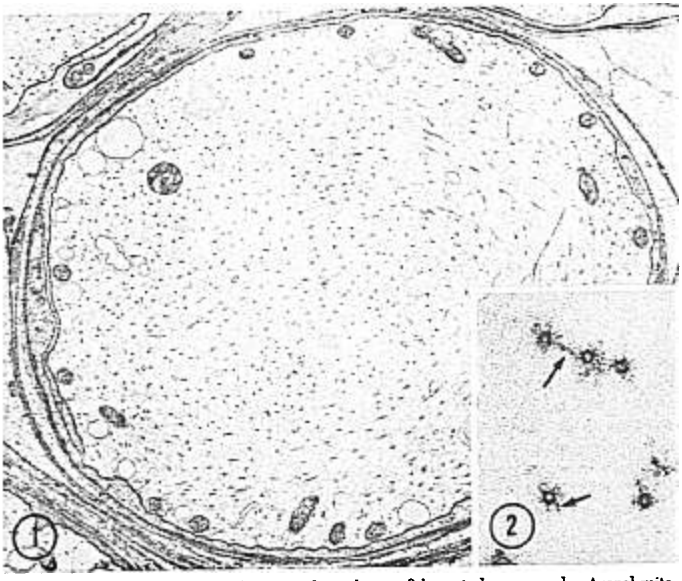

FIG. 1. Cross-sectional view of an axon from the crayfish ventral nerve cord. Axonal mitochondria are located near the periphery of the axon. Microtubules appear as small dots scattered throughout the axoplasm. Several microtubules have been sectioned obliquely at the upper right. ×9,500.

FIG. 2. Wispy filaments (arrows) radiate from the surface of the microtubules into the surrounding axoplasm. The upper arrow shows filamentous material connecting two adjacent cross-sectional microtubules. ×94,900.

see Fink *et al.*, Kennedy *et al.*, this volume, pp. 422–443.) It is not known whether these differences in stability relate to small differences in the tubules themselves or to differences in the ensheathing material. The variable stability of MT in response to cooling, warming, pepsin digestion, and colchicine led Behnke and Forer[10] to suggest that at least four classes of MT can be distinguished by these means. The variation in stability applies also to MT in different axons, even though these MT are morphologically indistinguishable. For example, colchicine depolymerizes MT in the rabbit vagus fibers,[7] but has no readily apparent effect on the morphology of the MT in crayfish ventral-cord axons.[9, 11]

Microtubules are composed of a protein which has some similarity to actin, a major protein in muscle. The protein which makes up the microtubules is remarkably uniform in all microtubules, even those from very diverse types of cells.[2] The individual molecules of the protein, called the "subunits," are held together to form the tubular shape by forces that are not very well understood. These forces are not strong, however, and this accounts in part at least for the general instability of the tubules. The geometrical positioning of the subunits to form a tubule results in 12 subunits' making one perimeter.

The microtubular protein, "tubulin," can be isolated in reasonably pure form. Many of its

physical properties have been determined.[12-14] Tubulin has an unusual property, in that the drug colchicine attaches to it and in so doing can prevent the tubulin molecules from forming normal microtubules. This binding of colchicine to the microtubule protein is the molecular basis for many, if not all, of its pharmacologic actions. A number of other important drugs, such as the antileukemic agent, vinblastine, also interact with tubulin, and these interactions are critical to the drug action. As pointed out, microtubules are major components of the mitotic apparatus in dividing cells. Many antimitotic drugs are effective through interaction with tubulin, thereby preventing cell division.

Numerous functions have been suggested for microtubules or microtubular systems. Suggestions range from the very speculative to the reasonably secure. There is reason to believe that the MT are continuous over a long stretch of axons or dendrites. One provocative idea, which takes this into account, proposes that MT act as a communication system, conducting "signals" from one place to another within the cells. Microtubular arrays are common in sensory receptors,[2] and they may be intimately involved in sensory transduction. It should be kept in mind that there is little direct experimental evidence bearing on the function of MT, and also that the various theories are not mutually exclusive. One aspect is clear: they are not directly involved in the electrical conduction of nerves.[7]

Most cell biologists believe that microtubules function primarily as a cytoskeleton, a skeleton within the cells and responsible for the individual shapes of cells and the rigidity of elongated cellular processes. Indeed, it has been shown that integrity of the microtubules is necessary for the normal shapes of many cells. A second view is that microtubules or microtubular systems are concerned with the coupling of metabolically produced chemical energy to mechanical events. In other words, "chemo–mechanical energy couplers." The reason for this view is their occurrence in motile systems such as flagella, cilia, and sperm tails. For the nervous system the movement of materials from the cell body out and along the axons, axoplasmic transport, may be a special case of coupling metabolic energy with a mechanical event. Work from many laboratories has shown that the integrity of the axonal microtubules appears to be vital to axoplasmic transport.[11, 15, 16] The papers on the effects of anesthetics on axonal transport in this volume are relevant to this point.

The involvement of MT in the movement of materials in cytoplasm is inferred from studies which demonstrate 1) the physical relationship of cytoplasmic constituents, such as pigment granules, to microtubules during the movement of the granules, and 2) the inhibition of these movements by agents which disintegrate microtubules (such as vinblastine) or interact with the protein, tubulin (such as colchicine). The transport of pigment granules in melanocytes is sufficiently similar to the transport of neuronal constituents that some lessons can be taken from the detailed studies of the melanocyte. The conclusion from these studies is that the MT are defining the channels, and possibly the motive force, for the movements of the granules.[17, 18] In axoplasmic transport certain cytoplasmic constituents are moved from the neuronal cell body along the axon at several velocities. There is a "slow" velocity of about 1 mm/day, which appears to be universal in all nerves, and there are faster velocities which are less uniform among axon types and particular cytoplasmic constituents. The papers in this issue which report the effects of halothane and lidocaine on axonal transport refer to the "rapid" movement of proteins in the rabbit vagus nerve, which has a velocity of about 400 mm/day. The transported material is not the bulk of the cytoplasm flowing; rather, it is a transport of selected constituents. The driving force for the transportation is generated locally; that is, transport will go on in isolated segments of axons.[19, 20] A more detailed role of the microtubules in axoplasmic transport is not known. The possibility that microtubules are involved in the translocation of materials is supported by the results of research which have implicated them (or the tubular protein itself) in the release of secretory products such as thyroxin, insulin, and adrenalin.[21, 22]

It should be noted that tubulin may not be present in MT only, but may also be a component protein of membranes.[23, 24] The likelihood that tubulin is part of the biochemical

machinery for release of neurotransmitters from neuronal terminals is interesting. Again, there might be relevance to anesthesia if anesthetics interacted with membrane-held tubulin to interfere with the release of transmitters at synapses.

Early investigators found more than 40 years ago that volatile anesthetics could inhibit cell division [25] and alter the sol–gel state of cytoplasm.[26] More recently, nonvolatile anesthetics have been shown to inhibit cell division of cultured mammalian cells.[27] Halothane causes metaphase arrest of the mitotic apparatus [28, 29] and retraction of heliozoan axonemes [30] and induces ciliastasis in *Tetrahymena*.[28] Since microtubules are a major structural component of mitotic spindles and cilia and are found in highly ordered arrays in heliozoan axonemes, these observations suggest that anesthetics may alter the structure of microtubules and subsequently impair their function. Observations similar to these, coupled with the observation that microtubules are the most numerous organelle in axonal ultrastructure, led Allison and Nunn [31] to postulate microtubular disruption as a possible basis of anesthesia. To account for the production of narcosis, they assumed that microtubules play a role in impulse transmission. However, microtubular disruption by colchicine [7] or vinblastine [11] has little effect on impulse conduction in isolated nerves. Sufficiently high concentrations of halothane disrupt axonal microtubules of rabbit vagus nerves, but lower concentrations of halothane (still higher than those used clinically) have little effect on microtubular structure and may actually increase the number of microtubules per unit of axon area.[7] In crayfish nerve cords halothane causes a structural transformation of axonal microtubules into a larger "macrotubular" form.[32] Several volatile anesthetics, including halothane, induce reversible changes in the fundamental structure of globular proteins,[22, 24] and we suggest that these macrotubular forms are derived from microtubular protein by a similar mechanism. Recent work by one of us (R. E. H.) on isolated microtubular preparations has provided evidence that halothane can directly affect microtubular structure.

It is against this background that the articles about the effects of halothane and lidocaine on axonal transport in this issue should be considered. Studies such as these may ultimately elucidate basic cellular events needed to explain the cytopharmacologic basis of anesthesia, and should provide additional insight into the role of a specific cellular organelle, the microtubule, in the fundamental processes of cell function.

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