PHARMACOLOGY OF DRUGS THAT AFFECT INTRACELLULAR MOVEMENT

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Frederick E. Samson
Ralph L. Smith Mental Retardation Research Center, University of Kansas Medical Center, Kansas City, Kansas 66106

1 INTRODUCTION

Although the movements of cellular constituents on first glance often appear to be random in character, they are more often highly controlled, and the concept that emerges is that the cell is organized even at the molecular level. Indeed, a function of intracellular membranes, granules, vesicles, and organelles is to segregate functionally related molecules. Further, if these assemblies are scrambled, the cell has a considerable capability for sorting them out again. What this amounts to is a segregation of "unit processes" into increasing levels of organization and performances. To coordinate these levels, the traffic within the cell must move under a high degree of control.

In a recent review, Allen (1) classifies cytoplasmic movements into three basic types: (*a*) bulk cytoplasmic transport that accompanies pseudopodia formation and ameboid locomotion; (*b*) bulk cytoplasmic transport that is unrelated to cell locomotion and serves to circulate cytoplasm; (*c*) selective transport of cytoplasmic constituents (such as nuclear movements) unrelated to any bulk transport. Allen points out that the endoplasm of cells is variably resistant to the displacement of particulates within it, behaving as a gel of cross-linked polymers undergoing sol $\rightleftharpoons$ gel transformations.

This review focuses on the selective transport of cytoplasmic constituents in neuronal processes, for the neuron has proven to be suitable for the study of the action of pharmacological agents on these movements. Pharmacological research has contributed substantially to the understanding of these intracellular events, and in turn such studies have shed light on the mechanism of some drug actions.

2 THE NEURON AS A MODEL

Neurons as a class of cells have a great range in size, from 20 μm to greater than several meters long (in the whale for example) and a corresponding heterogeneity

of shapes. They are characterized by a high degree of functional segregation (2) with the spatial separation for example of impulse reception, action potential conduction, and synaptic transmitter release. Such functional segregation is not unique to neurons of course, but the segregation of particular importance here is the almost complete restriction of protein synthesis and many types of organelles to the perikaryon. The axon itself is entirely lacking in ribosomes and with increasing distance from the cell body the ribosomes selectively disappear in dendrites (2). Therefore, those neurons with long axonal processes, which depend upon components manufactured in the perikaryon, make accessible for study the traffic that moves within them. These movements of cytoplasmic components within the neuron are referred to as axoplasmic transport (AXT) or less correctly, axoplasmic flow, and more correctly as neuroplasmic transport. Although the neuron is specialized in its own way, it seems reasonable to assume that the general principles of intracellular movement (ICM) in neurons are essentially the same for all eukaryotic cells.

3 NEUROPLASMIC TRANSPORT (AXOPLASMIC TRANSPORT)

3.1 Anterograde Neuroplasmic Transport (Away From the Cell Body)

There are a number of excellent reviews of axoplasmic transport (3–9) and an overview of the effect of drugs upon axoplasmic transport (10). The movement of axoplasmic components from the cell bodies to the terminals is an old concept but the classical studies of Weiss & Hiscoe (11) gave the first direct demonstration. A major breakthrough was provided by Droz & Leblond's demonstration that the movements of proteins could be traced by labeling them with radioactive precursors (12). Recently a number of nonradioactive markers have been developed. The subject may be summarized as follows: a wide variety of transported materials have been identified (enzymes, proteins, phospholipids, catecholamine-containing granules, glycoproteins, 5-hydroxytryptamine, dopamine-β-hydroxylase, etc). The most extensively studied are labeled proteins, some of which move at rates in the order of 1 mm/day (slow transport) and another group that move about 400 mm/day (fast transport). However, there is a wide spectrum of rates reported for various types of cytoplasmic components (see reference 5 for summary). Among the most rapidly moving are the neurosecretory granules of the preoptic nucleus, calculated to move 2000 mm/day (13).

The transport processes bring not only materials for maintenance and renewal of the axon, but also some of the molecules released at the terminals. Recently the important trophic action that nerves have on striated muscles was shown to be related to axoplasmic transport (14, 15).

The movements of intracellular particles have been studied in a wide variety of cells with specialized light microscope techniques, and there is a large literature on the subject (16–18). There is an increased interest recently in the use of dark-field microscopy and the optical sectioning quality of Nomarski optics in observing the movements within axons (19, 20), and computer analyses of the movements have been carried out (21). The particles visualized by these techniques appear to move in channels in a saltatory fashion. They move in directions both toward and away

from the cell body (see section 3.2 below). The particles have a range of velocities with the fastest ones moving at speeds comparable with fast axonal transport (FAXT). This method is limited in that only the particles visible can be studied, but it provides another very important window to the events of intraaxonal movements.

3.2 Retrograde Neuroplasmic Transport (Toward the Cell Body)

In Section 3.1, the movements of neuronal constituents synthesized in the cell body out to the terminals are briefly summarized. Probably as important and extensive as the anterograde movements is the traffic of axoplasmic constituents toward the cell bodies. Indeed, the particulates visible with Nomarski microscopy that move retrograde outrumber those that move anterograde (20). It has been recently recognized that the retrograde intraaxonal transport provides a major route for substances from the periphery to the central nervous system. A wide variety of macromolecules, including proteins exogenous to the neuron, as well as some viruses, move to the cell body from the terminals (22–24). The rate of these movements is in the order of 75–90 mm/day. Research has been carried out with markers such as horseradish peroxidase, which are taken up by pinocytosis into axon terminals and move intraaxonally, and have great potential for studies of this interesting transport (25, 26). The exciting concept that this retrograde transport may serve as a communication mechanism between the terminals and their microenvironment and the genetic apparatus in the cell body has been supported by the recent demonstration that nerve growth factor is specifically transported toward the cell body in adrenergic neurons (27).

4 MECHANISMS OF INTRACELLULAR MOVEMENTS

The mechanism(s) underlying ICM are no doubt the work of complicated machinery. Most of the ICM are not driven by diffusion and thus require metabolically generated energy. Therefore, drugs that interfere with the flow of free energy in the cell generally will arrest the movements. For example, FAXT depends on a local supply of energy: anoxia, dinitrophenol, and other energy flow inhibitors have been shown to inhibit FAXT (19, 28–30).

There are two major considerations with regard to the ICM mechanisms: one, what are the chemomechanical energy–coupling components that harness metabolically generated energy to drive them and, two, what are the components that give specificity such that certain cytoplasmic constituents are moved to particular intracellular locations? The implicated structures are extended surfaces such as the plasma membrane; tubular organelles such as the endoplasmic reticulum and microtubular channels; and linear fibrillar elements such as actomyosin, microfilaments, neurofilaments, and microtubules (external surface of MT). It is likely that each plays a part in some of the various types of ICM.

4.1 Plasma Membrane

The movements of the plasma membrane and in particular the peristaltic waves, which were shown by Weiss to sweep down the axonal membranes (9), have been

proposed to have a role in ICM. There are reasons, however, that might rule out this proposition. The movements observed in "skinned" amoeba (plasma membrane removed) by Allen and his colleagues show that at least the class of ICM studied required neither a plasma membrane nor hydrostatic pressure generated by an ectoplasmic tube (1, 31). In myelinated nerves, the analysis of axoplasmic movement and myelin movement revealed no evidence to support the idea that axoplasmic flow results from peristalsis in the myelin covering the axon (32). Further, it is difficult to see how peristalsis could provide the specificity or sufficient intracellular hydrostatic pressure for the movements within the axoplasm of, say, large invertebrate axons.

4.2 Endoplasmic Reticulum

In some cell localities, proteins, lipids, and probably other materials are known to be distributed through the endoplasmic reticulum (ER). These substances may accumulate within the ER and, in addition, may be modified while within the ER. The path of secretory material from the polysome to the lumen in exocrine cells and the packaging of material for transport involve the ER. The movement of protein and other constituents within the ER is a major subject in its own right and from the viewpoint of future research, pharmacology could give insight into these important transport events. The smooth ER frequently seen in axons may be involved in axoplasmic transport but no clear-cut hypothesis has emerged that is backed by any compelling experimental evidence.

4.3 Microtubule Channels

The internal diameter of microtubules (MTs) is about 15 nm and could accommodate sizable molecules. When appropriately stained, dense particles, which some authors have interpreted as macromolecules, are revealed in the central core of the MT (33). Whether this is a stationary structure or material in transit is unknown. (See section 7.)

4.4 Actomyosin

Actin has been shown to exist in a wide variety of nonmuscle cells (34) and is probably a universal constituent of eukaryotic cells. The properties of the actin in nonmuscle cells are remarkably similar to those of muscle actin, and this actin is probably involved in the cellular movements, ICM, and other mechanical events. Where contractile systems are known in detail an interaction of actin with myosin is involved. Myosins, in contrast with actins, are more diverse in their properties. It is now clear that myosin also is present in a number of nonmuscle vertebrate cells (34). Actomyosin complexes have been identified in brain (35). Further, the related protein tropomyosin has been identified in nervous tissue (36). An attractive, unifying concept is the idea that actomyosin is universal in eukaryotic cells and, with some individual modification or specialization, underlies most if not all of the movements, both cellular and intracellular. The evidence is compelling although the details of the activation of the force generation and exertion are less well known in nonmuscle cells than in striated muscle (1, 34). The extensive studies in protozoa emerge as almost conclusive that many of the intracellular movements are driven

by an actomyosin system (16). In particular, the class of ICM described as bulk cytoplasmic transport by Allen (1) is probably motivated by actomyosin.

4.5 Microfilaments

Microfilaments are identified as a class primarily on the basis of their size (about 4–5 nm). They are a heterogeneous class; for example some are clearly actin-like (bind heavy meromyosin) and some are not, and some are cytochalasin B sensitive (see Section 6). Those that are actinoid probably are part of an actomyosin system.

4.6 Neurofilaments and Other 10 nm Filaments

The 10 nm filaments may be a distinct class of filaments that occur in a number of types of cells. The neurofilaments are typically abundant in myelinated axons and less abundant in unmyelinated axons, and the component protein has been characterized by Davison (37). From time to time the suggestion is made that the neurofilaments are involved in axoplasmic transport; however, there is no evidence as to the function of any of the 10 nm filaments. If a reasonably specific pharmacological agent that interacts with those filaments were found, it would give a major impetus to understanding their function as colchicine has done for microtubules.

4.7 Microtubules

Microtubules are proteinaceous, intracellular tubular structures that are found in varying numbers in almost every type of animal and plant cell. They have a characteristic tube structure with an outside diameter of 25 nm and an inside diameter or channel of 15 nm. They are abundant in neurons, where they may run unbranched in dendritic and axonic processes for several micrometers. They are the principal fibrous protein of the mitotic spindle and dominant components of cilia and flagella. They often occur in highly ordered arrays. Indeed, as methods for ultrastructural studies improve it seems likely they will be revealed as components of larger systems in which a number of MTs are linked and function in a coordinated way. Since the widespread use of glutaraldehyde as a fixative for electron microscopy (which preserves MT structure better than previous fixation methods do) and the discovery that colchicine binds specifically to tubulin, a component protein of MTs, interest in the MT as an entity for research has greatly increased. This research has enlarged the understanding of many cellular functions, and there are a number of excellent reviews that cover the various aspects of the biochemistry (38, 39) and biology (40, 41) of microtubule protein.

Among the functions proposed for MTs, which now constitute a sizable list, the most convincing has been their role in the development and maintenance of an isometric cell form (42) and in intracellular movements. The effects of the pharmacologic agents colchicine and the vinca alkaloids have played a leading role in these findings. The evidence implicating MTs as a necessary component of the biological machinery that moves chromosomes and other cytoplasmic constituents in Allen's class 3 type of movements may be outlined as follows: the MTs are often oriented in the right way for transport, especially in extended processes such as axons; their location with respect to large cellular inclusions (such as pigment granules and mitochondria) reveals that they at least act as guides, if not directly,

in the propulsion; their characteristic, universal presence in motile processes such as flagella and sperm tails suggests that chemomechanical energy coupling is part of their physiological function. But among the best arguments are the findings that pharmacological agents that have a strong affinity for the MT protein subunits, and in many cases that disrupt MTs, arrest cytoplasmic translocations (see section 5).

Although a few papers have questioned the essential role that MTs have in ICM, they are not persuasive enough to outweigh the contrary evidence. One paper (43) is weakened by the small number of observations (of two animals) and by the sampling problems of electron microscopy. In another study the shuttling movements of the kinetocysts in heliozoan axopods, and the movements of nonmotile bacteria adhering externally to the axopods were analyzed, and it was concluded that the plasma membrane of the axopods rather than the MTs mediates these movements (44). The different average velocities and variances of the inward movements contrasted with the outward, suggested two fundamentally different mechanisms for "in versus out" (as in the case of the melanophores where inward and outward movements of the granules are differentially sensitive to drugs pointed out in section 6). When the MTs were destroyed by colchicine, similar movements persisted but were not in straight lines. This implies that the MTs do not provide the impelling force but rather the directionality. It is relevant that actin filaments (at least in cultured neuroblastoma cells) tend to be a network immediately beneath the plasma membrane (45) and may provide the driving force rather than the plasma membrane itself, which would not seem to have sufficient mass to accomplish such a large mechanical energy requirement.

Given that MTs are involved in transport, the question is "how." The actinoid character of tubulin has not been overlooked and it has been proposed that the mechanism is similar to the sliding filaments of actin and myosin of muscle contraction, with tubulin behaving akin to actin, interacting with a myosinoid protein associated with the particulates (46). Bridges directly connecting vesicles and MTs have been illustrated in several preparations (47).

Another mechanism that has been proposed takes into account the layer of anionic polyelectrolyte, which ensheaths axonal MTs and also extends to form a network through the axoplasm (48). This material is associated with MTs in axons and stains with alcian blue (49), ruthenium red, alkaline bismuth (50), and lanthanum (51). These methods under the conditions used have some specificity for mucopolysaccharides, or at least for polyanionic molecules with high charge density. In this minimal model, the MTs are the anchor points of a network composed of macroanionic molecules (macroions). The branching molecular units would specifically attach to the transported molecules or organelles. It is well known that linear flexible macroions characteristically extend and contract with small ionic changes in their immediate environment (52). Indeed, polyelectrolyte gels can be made to act as transformers of chemical energy to mechanical work (52). The expansion-contraction movements of the anionic polyelectrolyte network might be expected to be discontinuous and generate the saltatory character of cytoplasmic movements. A dynein-like protein with ATPase activity has been reported to be associated with neuronal MTs (55).

5 ANTIMITOTIC DRUGS

Certain antimitotic drugs are now known to block mitosis by interfering with the movements of the chromosomes. Further, the molecular mechanism of the blockade is the disruption of the microtubules of the mitotic spindle by the binding of the drug to the microtubule protein (53). The drugs for which this mechanism is best established are colchicine and some of its derivatives, the vinca alkaloids (vinblastine and vincristine), and podophyllotoxin. The importance of these findings should not be underestimated for they have led to substantial advances in a wide spectrum of research from biochemistry to medicine. The escalation of knowledge about the microtubules is an example. The interaction of these drugs with microtubule protein has been comprehensively reviewed in reference 54.

5.1 Colchicine

Because colchicine binds specifically to tubulin, the protein subunit of the spindle microtubules, it can be considered a prototype of a class of antimitotic drugs known as spindle poisons (56, 57). The details of the interaction of colchicine with tubulin are covered in reference 54.

Colchicine blocks ICM in addition to those of chromosomes in a wide variety of cells. It has been shown to block the axoplasmic transport of axoplasmic constituents such as acetylcholinesterase in sciatic nerve (58), amine storage granules (59, 60), proteins (61–63), and sulfated mucopolysaccharide protein (64). Further, colchicine blocks the movements of pigment granules in chromophore cells (65), nuclear migrations in fused cells (66), in other words, a wide variety of cytoplasmic movements. Although some work suggests that colchicine blocks only the rapidly transported axonal constituents, a greater number of studies show that the slow transport (1 mm/day) is also blocked (7, 61, 63, 67).

Colchicine also blocks the retrograde transport of horseradish peroxidase (26), nerve growth factor (27), and optically observed particulate movements (19). In many preparations the blockade of transport is associated with a disappearance of the microtubules. The specific binding of colchicine to the microtubule protein and the microtubule disruption has been the major contributing evidence to the conclusion that microtubules are an essential component of the biological machinery for ICM.

An increasing variety of cellular responses affected by colchicine (and also by the vinca alkaloids) are appearing in the literature. Included in these are the glucose-stimulated release of insulin (68); nerve-stimulated release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves (69); TSH-stimulated release of thyroxin (70); acetylcholine-stimulated release of catecholamines from the adrenal medulla (71); release of histamine from mast cells (72) and leukocytes (73); release of growth hormone and prolactin induced by prostaglandin E (74). The inference drawn from these experiments generally is that the MTs are involved in the mechanical movements leading to release of the secretory products contained in granules. Still other intracellular events such as the conversion of proparathyroid hormone to parathyroid hormone (75) have been shown to be influenced by colchicine (and

the vinca alkaloids). The *stimulation* of steroid secretion by colchicine and other antimicrotubular agents has been interpreted by Temple & Wolff (76) to mean that access of cholesterol to the mitochondria is ordinarily restricted by the MTs and that stimulation occurs when this restriction is removed by the antimicrotubular agents.

The antimicrotubular agents (but not cytochalasin B, which does not bind to MT) have been shown to affect plasma membrane topographical responses (77). These agents reverse the inhibition by concanavalin A of patch formation and immunoglobulin receptor mobility in lymphocytes (78). Also, lymphocyte mitogenesis induced by the binding of antigen to the cell surface receptors is inhibited by colchicine at a step prior to spindle formation. It is not known whether the colchicine-binding protein in this case is tubulin, but the similarity of effects of a variety of antimicrotubular drugs suggests that it is. The hypothesis proposed by Edelman postulates a subsurface protein (tubulin?) in association-dissociation equilibrium with certain cell receptors that anchors the receptors and modulates their mobility. Further, he proposes that this protein assembly may be involved in a signaling process for mitogenesis (78).

The question of the specificity of colchicine for microtubule protein in some responses continues to be raised (79). In an attempt to resolve this, Zweig & Chignell (80) have suggested that a stronger case for the participation of MTs in these processes could be made if the effect were directly related to the ability of a given analog to bind to tubulin. To illustrate their approach, they have compared the ranking of the binding of seventeen colchicine analogs, podophyllotoxin, and vinblastine to tubulin with the ranking in the mouse sarcoma test and in anti-inflammatory activity (80). They found a good correlation between the binding of these drugs and their efficacy as both antimitotic and antigout agents. This supports the theory of Malawista (81) that the binding of colchicine to tubulin is responsible for its antigout as well as its antimitotic activities. The lumicolchicines are also useful tools for such studies since these derivatives do not bind to tubulin (54) and are not antimitotic. Lumicolchicine does not affect FAXT under conditions where colchicine does (82).

The blockade of transport by the tubulin-binding drugs is sometimes reported as occurring without detectable changes in the ultrastructure of MTs. However, the sample of tissue in electron microscopy is small and flaws in the MTs could escape attention. Furthermore, the ultrastructural appearance of the MTs may not always be a reliable index of their functional integrity. This would be particularly true if the dynamic polymerization-depolymerization of pool subunits and MTs is necessary for functioning. Colchicine binds to the tubulin subunits and not to the polymerized MT itself, so it causes the disappearance of the MT only when there is an ongoing polymerization-depolymerization by drawing the equilibrium away from the polymerized state.

5.2 Vinca Alkaloids

The interaction of the vinca alkaloids with tubulin depolymerizes the microtubules and gives rise to highly regular crystalloid structures (83). The binding site of the vinca alkaloids on the tubulin molecule is different from the colchicine site. The chemical properties of the vinca alkaloids that bind to tubulin and the crystalloids

that are formed have been studied in some detail (40). However, it should be cautioned that the vinca alkaloids interact and can precipitate a number of acidic proteins and nucleic acids in addition to tubulin (84). In particular, the vinca alkaloids interact with actin. Thus, the vinca alkaloids appear to be less specific than colchicine. Most of the studies on axoplasmic transport are with vinblastine sulfate and vincristine sulfate, and there is little question that they are potent blocking agents—more so than colchicine—on both the fast and slow types of axoplasmic transport (85, 86), and the retrograde transport as well (87).

The antimitotic activity of several of the vinca alkaloids (mitotic index 50%) is about the same for vinblastine sulfate, vincristine sulfate, and desacetylvinblastine sulfate (7.5×10^{-8} M), while leurosidine is about 100 times less potent. The upper indole moiety of the dimeric alkaloid, catharanthine is a weak antimitotic, while the lower indole moiety, vindoline, is inactive (54). It is noteworthy that the interaction of these derivatives with tubulin parallels the antimitotic activity (54).

5.3 Podophyllotoxin

Another plant aklaloid that arrests mitosis in a similar manner to colchicine is podophyllotoxin. This drug binds to tubulin at the same (or greatly overlapping) site as colchicine, but with somewhat different binding forces (54). Picropodophyllotoxin, an isomer of podophyllotoxin, is reported to have less affinity for tubulin and correspondingly is a weaker antimitotic agent than podophyllotoxin (54). Podophyllotoxin has an inhibitory effect similar to that of vinblastine on the release of iodine from the thyroid stimulated by the thyroid-stimulating hormone (70) and on catecholamine release from the adrenal medulla (71).

5.4 Griseofulvin

Griseofulvin, a product of mold metabolism, is a fungistatic agent that resembles colchicine in its structure and in its antimitotic action. However, its mechanism of action appears to be different since it does not interact directly with tubulin, nor does it block polymerization of MTs in vitro (54). There is evidence that it disrupts the relative position of the microtubules with respect to one another similar to the action of isopropyl N-phenylcarbamate. The possibility has not been tested that it may interact with the "mucopolysaccharide" material ensheathing and connecting the microtubules: the "microtubular network" described in section 4.7 above. Griseofulvin blocks the granule movements in melanocytes (88) and this is rapidly reversible when the drug is removed.

6 CYTOCHALASIN B

The cytochalasins are a group of mold metabolites. One of these, cytochalasin B, (cyto-B) has effects on a variety of cellular activities and has been importantly exploited by Wessels and his colleagues (89). The cytochalasins inhibit a variety of cellular movements (cytoplasmic streaming, cytokinesis, pinocytosis).

The site of action of cytochalasin B seems to be on a class of microfilaments. It does not change the microtubules or neurofilaments in any discernible way. Its

effects on axoplasmic transport are not clear-cut. It blocks both the slow (1 mm/day) and a faster (10 mm/day) protein axoplasmic transport in a crayfish ventral cord preparation (90). Also the drug caused a disruption of the "constrainment" of the faster transported protein in the crayfish preparation, which suggests that the cyto-B–sensitive microfilaments may be important to the directionality of the transported materials. On the other hand, there are reports of no effect of cyto-B on axoplasmic transport. For example, it had no effect on the transport of norepinephrine storage granules in adrenergic nerves (60). The drug is poorly soluble in aqueous solutions so the delivery of the drug may account for some of these disagreements. Cytochalasin B inhibits the release of catecholamines and dopamine-β-hydroxylase from adrenergic terminals (69). Further, it inhibits the outward movement of granules in the melanocyte, which colchicine does not; cyto-B does not affect the inward movement of the granules as colchicine and vinca alkaloids do (88).

The intracellular translocation of pigment granules in the dendritic arms toward the cell centers in the chromatophores of the glass shrimp (*Palaemonetes vulgaris*) is not inhibited by colchicine or vinblastine. (Yet the MTs are gone and vinblastine-tubulin crystals appear.) However, this pigment movement is reversibly blocked by cytochalasin B but with no discernible loss of microfilaments (91). Thus centripetal pigment movement in this preparation does not require MTs but some cyto-B-sensitive element. This action is in contrast to the actions of these drugs on the granule movements in the melanocyte described above. Although cyto-B clearly disrupts actin thin filaments in some preparations, there are other preparations that argue against the actin filament as the target molecule. It has been pointed out in a recent discussion of cyto-B actions by Pollard & Weiking that the dose-dependent effects indicate more than one target molecule (34). Indeed, there are reasons to believe that the cyto-B action is at the cell surface and the changes in microfilament structure are secondary (92).

7 ANESTHETICS

The effects of anesthetics on AXT have been valuable in sorting out the immediate relationship of electrical excitability of neurons from AXT.

7.1 Halothane

The effect of the general anesthetic, halothane, on ICM has been studied in several types of preparations. On the strength of the observation that the axopods of *Actinosphaerium* (the axopods are dependent upon MT integrity) retracted during exposure to halothane, Allison & Nunn proposed that the mechanism of the anesthesia is via the disruption of MT (93). More direct studies on halothane anesthesia have shown that this hypothesis is not correct (94). Concentrations of halothane that produce anesthesia do not affect axoplasmic transport (95) nor are the microtubules discernibly affected in vagus nerve axons (94). Much higher concentrations of halothane inhibit axoplasmic transport (95), reduce the number of microtubules, and disrupt their morphology (96).

7.2 Local Anesthetics

The effect of several of the common local anesthetics on axoplasmic transport has been studied. Fink and colleagues examined the effect of lidocaine on the fast axoplasmic transport of protein in rabbit nerves in vitro. Concentrations in the order of 0.2% slowed the transport reversibly, while concentrations in the order of 0.6% completely blocked transport with slight reversibility and also caused a disappearance of axonal microtubules. They later showed that the block of impulse conduction and axoplasmic transport changes did not correlate temporally (97).

Other studies on local anesthetics involving lidocaine topically applied (98), ophthaine on an optic nerve preparation (99), and procaine (1%) on slow and fast transport of fibers in the sciatic nerve emerging from the ventral horn cells (100) showed no effect. Lidocaine and etidocaine under local anesthetic conditions did not change the rapid transport of catecholamine-synthesizing enzymes in sciatic nerve (101). Thus it appears that local anesthetics at anesthetic concentrations do not directly influence axoplasmic transport. It is worth noting that the electrical excitability of nerves is not directly dependent upon the MTs. That is, vagus nerve fibers treated with colchicine under conditions that extensively reduced the number of MTs did not show any changes in the rates of action potential conduction or amplitude (94). Also, neither the replacement of Na^+ in the medium with an equivalent amount of K^+, which leads to a depolarization of the nerve, nor indeed the replacement of Ringer's solution with an isotonic sucrose solution influences the fast axoplasmic transport (102).

8 NEUROPATHOGENS

A wide variety of agents and diseases have been described as neuropathogens. There are good reasons for believing that some of these that are associated with neurofibrillary pathology have among their effects the disruption of axoplasmic transport.

8.1 Aluminum Salts

Aluminum salts have been used experimentally to induce a neurofibrillary degeneration, and this preparation has been used as an experimental model for neurofibrillary diseases (103). In a study of axoplasmic transport in aluminum-treated rabbits (0.1 ml 1% $AlCl_3$ injected into the cisterna magna) with the neurological signs of the aluminum-induced pathology, the authors concluded that the axoplasmic transport rate was not changed (104). This is a surprising result in view of the rather severe pathology described. However, examination of the data and the methods used make it clear that the experiments were not adequate. That is, as these experiments were designed the axoplasmic transport observed would be that in the remaining normal fibers, and the axoplasmic transport failure in the fibrillary degenerated neurons would not be resolved. The Al-induced neurofibrillary tangles are tangles of 10 nm filaments and do not appear to be associated with alterations in the MTs or the other organelles of the nerve cell body (105). It is an important experimental model because the neurofibrillary tangles induced a state similar to those seen in a number of major neurological diseases.

8.2 Acrylamide and Triorthocresyl Phosphate

Chronic administration of acrylamide induces a type of neuropathy in which one might expect disruption of axoplasmic transport to be implicated. The slow 1 mm/day AXT was reported to be disrupted in cats with acrylamide neuropathy but not in triorthocresyl phosphate neuropathy (106). On the other hand, Bradley and colleagues concluded that both the fast and slow axonal protein transport waves in cats suffering from neuropathies induced with vincristine, acrylamide, and triorthocresyl phosphate were normal (107). They also point out that the complexities involved in transport studies make interpretation of the data difficult. Again, the design of these experiments is such that the transport in unaffected fibers would dominate and poor transport would be revealed only if it were pronounced and not close to the site of precursor injection. It seems evident that a degenerated axon would fail to transport proteins. The question is whether the "causation" site of action of a given neuropathogen is on the transport mechanism. Obviously it would be valuable to study the relationship of axoplasmic transport perturbation to the neuropathogens with better-designed experiments.

8.3 Galactose Neurotoxicity

A galactose neurotoxicity in chicks is associated with tremors and seizures resulting from a general disruption of energy metabolism of the brain. FAXT in the optic nerves was studied in galactose-treated chicks, and the amount of FAXT protein was decreased about 30%. The authors attribute this reduction of transport to an inadequate supply of required energy (108).

9 NEUROTOXINS

9.1 Tetrodotoxin

Tetrodotoxin is a valuable experimental tool that blocks sodium membrane channels and thus arrests the axonal action potential conduction. It does not affect fast axoplasmic transport (100). This might be expected in view of the lack of immediate correlation between electrical excitability and axoplasmic transport (see section 7).

9.2 Batrachotoxin

Batrachotoxin blocks membrane excitability by way of an increased Na^+ permeability (109). It was recently found to block FAXT in micromolar concentrations (110). Increasing Ca^{2+} in the medium had a protective action. The mechanism of action is not known but is unrelated to the increased Na^+ influx because the block occurred in the absence of Na^+ in the medium. Nor was the ATP level sufficiently lowered by the batrachotoxin to account for the inhibition by interruption of available energy supply. A clue to the mechanism may be found in the observation that tetrodotoxin, which blocks the action of batrachotoxin on membrane excitability, also prevented its blockade of axoplasmic transport (110).

10 MONOAMINE OXIDASE INHIBITORS

The monoamine oxidase (MAO) inhibitors pargyline and phenelzine have unusual effects on fast axoplasmic transport (111). Rats were given (75 mg Kg^{-1}) pargyline daily for up to 7 days, and the FAXT in sciatic nerve was studied. The rate of the leading front of the transported protein in the control was about 550 mm/day (in the range of other studies), but the rate of the leading front in the pargyline-treated rats increased almost 4 times (2000 mm/day). The authors related this change in FAXT to a myopathy, which results from the pargyline treatment. Their report is brief but is important enough to deserve comment. The design of the experiments did not permit a discrimination of the following possibilities: (*a*) an increase in uptake and incorporation of the labeled precursor and hence an earlier release from the cell bodies of the labeled protein; (*b*) the faster front being composed of proteins other than the control FAXT proteins. Since these findings have a bearing on the trophic actions of neurons and possibily on certain myopathies, they warrant more study.

11 MICROTUBULE-STABILIZING AGENTS

11.1 Dimethyl Sulfoxide

A number of agents that stabilize MTS are known. To date the most powerful is the solvent dimethyl sulfoxide (DMSO). It has been used to isolate a greater number of polymerized MTS from brain (112). In vitro, polymerization of tubulin is more rapid with DMSO present in the medium, and at an optimum concentration of 10% the MTs are stabilized against the depolymerizing actions of cooling and ionic strength (113). The effect of 10% DMSO on FAXT in vagus fibers is a complete blockage which is reversible; at 2% or lower the DMSO does not discernibly affect the FAXT. The ultrastructure of the vagal fibers in 10% reveals a swelling of glial cells and some unmyelinated axons but shrinkage of other unmyelinated axons; the MTs are abundant and prominent. Even these dramatic ultrastructural changes are readily reversible (114). Owing to the wide variety of DMSO's actions, an interpretation of these results is not definitive, but these results are compatible with the idea that stabilizing the MTs (that is, depressing the depolymerization-polymerization dynamics) may render them nonfunctional in ICM.

11.2 Heavy Water

The hypothesis that stabilizing MTs makes them nonfunctional was put forth based on experiments with D_2O on mitotic movements (115). D_2O is known to strengthen the gel state of cytoplasm and also to affect the ionization of weak acids (116). D_2O may also immobilize the filaments extending from the MTs (described in 4.7), which may be important in the motion-generating machinery.

The antimitotic effect of D_2O is well known (117) and it reversibly inhibits melanin granule movement in melanocytes (118). D_2O (50→90%) blocks the FAXT of proteins in frog sciatic nerve (119) and the release of growth hormone and prolactin from the adenohypophysis (74). At least, the idea that the sequential

disassembly-reassembly of MTs is necessary for their function (115) is compatible with some of the observations. The subject is complicated in that D_2O facilitates some responses in which the catecholamines are released from the adrenal medulla (71).

12 NERVE GROWTH FACTOR

Nerve growth factor (NGF) is a polypeptide that modifies the maturation and metabolism of adrenergic neurons and is a regulating molecule. It has been intensively studied and its amino acid sequence is known. It is transported retrogradely in specific neurons (see section 3.2). Almon & McClure (120) studied the effects of NGF on AXT in sympathetic neurons and found that NGF increases the rate of the fast-transported protein but decreases the rate of the slow-transported protein, indicating a selective action. They suggest that NGF also has a role in the physiological regulation of axoplasmic transport.

13 CONCLUSION

The intracellular movements of cellular constituents are largely nonrandom, organized events by which molecules and higher order aggregates are relocated within the cell. Pharmacology has an important role in the study of the intracellular movements and in turn, pharmacological research of these events has contributed to the understanding of the mechanism of action of some drugs. The locations of origin or synthesis of cellular constituents are usually not the intracellular locations of function, and ongoing metabolism is associated with continuous translocation. Diffusion as a translocation mechanism is important primarily for small molecules and even with small molecules, diffusion needs to be supplemented with bulk streaming and cytoplasmic stirring. Thus, metabolically generated energy must be available and the chemical form of the energy must be converted to a mechanical event. An additional requirement for intracellular movement is of an informational kind: specific constituents are translocated to specific locations.

Those neurons possessing long axons are useful for the study of intracellular movements of the selective type (the type unrelated to bulk cytoplasmic movement). The neuron has a high degree of segregation of functions and the long axon makes many aspects of transport accessible for study.

Drugs that disrupt the generation or flow of metabolic energy will interfere generally with intracellular movements. The antimitotic drugs (colchicine, vinca alkaloids, etc) have been particularly valuable because they arrest a wide variety of intracellular movements in a large representation of types of cells and there is a compelling correlation between their affinity for tubulin, their disruption of microtubules, and their pharmacological actions. On the strength of such studies, the inescapable conclusion is that the ubiquitous microtubules are directly involved in many intracellular movements. The anesthetics have been useful in resolving questions about the direct relationship between electrical excitability and transport. The basis of anesthetic action does not seem to be a disruption of intracellular movements.

A number of aspects of intracellular movements are promising for future research of practical importance, for example, the trophic functions of neurons, neuropathies, intracellular regulation, and regulation of hormone release.

The long-standing reservations concerning drug specificity, and indirect versus direct actions can be minimized as illustrated in the case of the tubulin-binding drugs. That is, if the correlation between the binding affinities of the drugs to relevant molecules and the pharmacological actions follows a close ranking order, the conclusions about mechanism are strengthened.

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