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RETROGRADE TRANSPORT OF MACROMOLECULES IN AXONS

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INTRODUCTION

Somatofugal or anterograde transport in the axoplasm, of organelles and chemical substances necessary for synaptic function and maintenance of axonal constituents, has been extensively studied and subjected to several reviews during the past decade (1–3). Lubińska's description of bidirectional movements within axons (4) and the observation of an uptake at axonal terminals of protein tracers, which subsequently accumulated in the neuronal perikaryon, lent direct support to the hypothesis that axonal transport is also in the somatopetal or retrograde direction (5). Both protein uptake and retrograde transport have been observed in the peripheral, central, and autonomic nervous systems (6–8).

Horseradish peroxidase (HRP) has been the most commonly used tracer protein in these studies; during the last few years the retrograde transport method with HRP has provided a most powerful tool in the tracing of anatomical pathways in the nervous system (9, 10). Somatopetal axonal transport evidently provides a mechanism for the spread of certain viruses and toxins in the nervous system (7), but its neurobiological and pharmacological implications have not, as yet, been extensively explored. This article aims to review recent observations concerning mechanisms by which a substance may be taken up by axonal terminals at synapses and, subsequently, be transported to the nerve cell body in the somatopetal direction.

UPTAKE OF MACROMOLECULES AT SYNAPSES

In the central nervous system the so-called blood-brain barrier prevents a nonselective passage of various substances from the blood into the parenchyma. This barrier maintains an optimal homeostasis in the brain and protects the brain from harmful substances (11). In the peripheral nervous systems the perineurium and to some extent the endoneurial vessels also provide a diffusion barrier; however, distally, at the neuromuscular junctions, the terminals of motor nerve fibers end openly (12,

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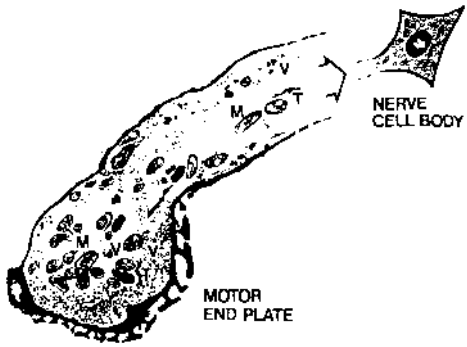

Figure 1 Schematic drawing showing the black reaction product of HRP outlining the axon terminal at a neuromuscular junction. HRP is incorporated into vesicles (V) of various sizes and tubular structure (T) and transported in the axon to the nerve cell body where accumulation can be seen even at the light microscopic level. M: mitochondria.

13). Thus, the protein tracer, HRP, injected into the blood, can directly reach the surface of the axon terminals at the motor end-plate, since HRP can pass the skeletal muscle vessels (14). Similarly, HRP can directly reach the axon terminal surface in the autonomic nervous system, e.g. in the iris following injection into the anterior eye chamber of the rat.

Axon terminals, especially in the peripheral and autonomic nervous system, may therefore be exposed to a variety of macromolecules from their surroundings. Thus, it is of fundamental importance to determine the mechanism by which a substance can attach to or be taken up by the axon, and to find out any specificity in the molecular incorporation. In recent years a number of observations have, in fact, indicated that axonal uptake of molecules may occur with a high degree of specificity.

Interactions Between Axon Surface and Exogenous Molecules

Of several tracer substances injected into the area of axon terminals, only a few can subsequently be detected in the nerve cell bodies (15). This probably implies that synaptic uptake of molecules is a very selective process. In this section some of the parameters that influence the attachment and uptake of a molecule to the axonal surface are reviewed.

NONSPECIFIC UPTAKE From studies of molecular uptake in fibroblasts, it is known that the molecular charge is important; a positive charge generally facilitates uptake. Thus protamine and histones are taken up more readily than albumin. Basic compounds, which penetrate easily, may also enhance the uptake of other substances, a so-called helper phenomenon. Therefore, basic polyamino acids can be used to enhance the penetration of a variety of substances such as toxins, polynucleotides, interferon, and viruses (16). The polycationic protein, protamine, induces a

nonspecific vesicular uptake of HRP into mouse skeletal muscle (17), but the possibility of enhancing neuronal uptake with this substance has not been tested.

Acidic carbohydrate groups are present on neuronal surfaces, where the periphery is heavily stained by the colloidal iron technique (18). This indicates the presence of acid glycoproteins in the glycocalyx at the outer surface of the plasma membrane (19). Glycoproteins have also been demonstrated in the synaptic gap substance by a variety of histochemical techniques at the ultrastructural level (20). These electric properties of the glycocalyx may explain why Bunt et al (21) found no accumulation in the nerve cell bodies of the acidic isoenzyme of HRP (isoelectric point pH 4.5), in contrast to the basic isoenzymes (isoelectric point pH 9.0) following injection into the area of axonal terminals in the rat visual system. However, any binding of HRP to cell surfaces does not appear to occur (22).

Another important parameter with regard to uptake is the size of the molecule. In general, the uptake increases with increasing molecular size (16). Uptake of bovine lactoperoxidase by mouse liver cells is much more rapid than that of HRP, which may be due to the greater molecular weight and higher isoelectric point of the former (23). However, in contrast to HRP, we have not been able to demonstrate any neuronal uptake and transport of lactoperoxidase or catalase in vivo. This, however, may be due to differences in sensitivity of enzyme histochemical techniques, since each enzyme requires an optimal fixation, substrate, and pH during incubation for its demonstration (24).

RECEPTOR BINDING Carbohydrate-containing macromolecules have also been localized to synapses by the technique of HRP labeling of lectins, e.g. concanavalin A (25, 26). Red blood cells coated with concanavalin A attach to axon membranes of chick sympathetic ganglia neurons, and these complexes move slowly in the retrograde direction at the surface (27). Lectin-HRP-receptor complexes are also incorporated into vesicles and tubules within 1 hr by isolated dorsal root ganglion neurons. Under the same conditions soluble HRP is not taken up (28), which corroborates with the observation that HRP is not incorporated from the surface of the neuronal perikaryon, but at endocytotic active axonal terminals [(29) see below]. Binding of lectins to receptors at the neuronal surface may therefore induce endocytosis analogous to the binding of lymphocytes to plasma membrane receptors.

That retrograde axonal transport occurs with a high degree of specificity of certain neurobiologically active substances has been investigated by Thoenen and co-workers. The selectivity of uptake can depend on properties common to all axon terminals; for example, tetanus toxin was taken up and transported in all neurons examined, while for other substances the uptake was restricted to certain populations of neurons; e.g. nerve growth factor was taken up by sympathetic and large dorsal root ganglion neurons but not by motor neurons (15, 30). A further example of such a selectivity in uptake with respect to the population of neurons is the transport of antibodies to dopamine-β-hydroxylase to sympathetic nerve cell bodies, but not in detectable amounts to either sensory or motor neurons (31). It may

therefore be suggested that membrane receptors exist at the axonal terminals and provide specific binding sites for these substances (32). The existence of receptors may also explain why certain viruses can directly attack neurons while others cannot (33).

NEUROTRANSMITTER UPTAKE The extensive literature on neurotransmitter uptake at synapses is beyond the scope of this review (34). Certain amino acid transmitters, however, can be accumulated into nerve terminals by a high affinity uptake process, and GABA and glycine, for example, are taken up by different populations of synaptosomes in homogenated rat spinal cord (35, 36). Furthermore, ^{3}H-glycine that is taken up by axonal terminals may be transported retrogradely to the nerve cell bodies (37). Differences in affinity of different neurons to different amino acids may explain why a retrograde amino acid transport has been observed in some studies (38), but not in others (39).

LIPOSOMES Liposomes can potentially be used as a carrier to introduce a variety of biologically active substances into neurons by uptake and retrograde transport. Liposomes form when water-insoluble polar lipids, phospholipids, are confronted with water (40). Substances including chelating and antiviral agents may be carried into cells after inclusion in liposomes. By changing the composition of the lipids in the liposomes, a selective affinity for neurons might possibly be obtained. However, only recently has the possibility of using liposomes in nervous tissue been explored. Some liposomes are highly toxic, while others are sufficiently benign to be used as carriers of therapeutic agents into the CNS (41). It should therefore be possible to use liposomes as carriers for antiviral substances and, by retrograde transport, to introduce them into sensory ganglia neurons or lower motor neurons, which often are the target for virus infections like herpes and poliomyelitis.

Mechanisms for Axonal Incorporation of Macromolecules

Because a significant mechanism for macromolecular uptake at synapses is by pinocytosis, this section is focused on recent observations of this process (42). Intact axonal membranes exclude passive diffusion of macromolecules into the axoplasm, but after an injury such a diffusion may occur. In addition, an active carrier-mediated transport exists for uptake of transmitters into neurons from extracellular spaces. This uptake occurs by the passage of single molecules through the membrane into the axoplasm probably without involving endocytosis (34).

MICROPINOCYTOTIC UPTAKE Several studies on invertebrate and amphibian neuromuscular junctions indicate that incorporation of HRP into pinocytotic vesicles at synapses can be increased by stimulating the synapses to transmitter release (29, 43, 44). In a study by Holtzman et al (72), when organotypic cultures of fetal mouse spinal cord were exposed to HRP in the presence of strychnine, which enhances transmitter release, HRP was incorporated into many vesicles at presynaptic terminals (29). On the other hand, when these cultures were exposed to HRP in the presence of Mg^{2+}, which depresses transmitter release, only a very few vesicles contained the tracer. In addition, in cultures exposed to HRP with strychnine-

induced stimulation for 1 hr, tracer accumulated several hours later in the neuronal perikarya. Markedly less HRP accumulated when the initial exposure to tracer was with Mg^{2+}. Concomitant with the accumulation of HRP in the perikarya, there was a loss of HRP from the axon terminals during the chase period, indicating that synaptic vesicle membrane turnover involves a stage of retrograde axonal transport to the neuronal perikarya and that the pinocytotic activity of a neuron occurs at the axon terminals, and not significantly at the cell body.

The ability of HRP to trace synaptic activity in nervous tissue in vivo has been shown in skate or frog retinal photoreceptors (45, 46). When exposed to HRP, photoreceptor terminals take up much of the tracer in the dark, while steady illumination markedly decreases uptake. The changes in HRP uptake with light varies in accordance with the expected activity in synaptic neurotransmission. HRP uptake appears to reflect activity in neurosecretory cells as well, as evidenced by the effect of castration on HRP incorporation into "synaptic" vesicles of the median eminence of the male rat (47).

Further evidence that increased uptake of HRP at synapses may be followed by an increased transport of the tracer up the axon has been obtained in studies on frog muscle nerve preparations (48). After exposure of the muscle compartment to HRP and stimulation of the nerve, an increased amount of HRP in the nerve compartment can be measured biochemically. Also cat lateral geniculate neurons accumulate less HRP from the projection area of the cortex within the first 24 hr following deafferentiation by unilateral enucleation, as compared to the other side with active synapses (49). These studies indicate that uptake and retrograde transport of molecules is directly related to the activity of synapses and transmitter release, an important observation when considering the possible implications of this phenomenon for neuronal function.

DIFFUSION THROUGH INJURED AXON MEMBRANE After a cell is injured, the permeability of a cell membrane rapidly increases, permitting leakage of macromolecules. Thus, after an axon is crushed or cut, HRP rapidly diffuses into the injured axon. It may subsequently be incorporated into organelles and transported up to the nerve cell body (50). Since injection of HRP into the innervation field of a neuron may involve direct trauma to the axon terminals, it may be difficult to decide whether HRP accumulated in the corresponding nerve cell bodies is the result of HRP uptake as an injured cell reaction or as a reflection of the activity of intact synapses. To establish that nerve cell body accumulation of HRP occurs by pinocytotic uptake at synapses, HRP was injected in very large doses intravenously. The tracer did not pass the cerebral vessels, except by pinocytosis around the arteriole (51), but this relatively small molecule (40,000 MW) passed through the vessels in skeletal muscles and diffused into the synaptic clefts reaching axon surfaces at the terminal at which incorporation into vesicles follows (14). The tracer could be seen in axonal organelles and after some hours of accumulation it appeared in the lower motor neurons in the brain stem and spinal cord (52). Thus, following this nontraumatic mode of injection, retrograde transport followed the physiological process of pinocytotic uptake at synapses.

RETROGRADE TRANSPORT IN AXONS

Direct observations on movement of organelles within axons in both directions were made in early studies on amphibian nerve fibers (53) and on nerve processes in tissue culture (54, 55), and, more recently, by the use of Nomarski interference microscopy on excised mammalian nerves in incubation chambers (56, 57). Evidence for a bidirectional movement of materials in axons has also been obtained from observations of an accumulation of enzymes, radiolabeled substances, and organelles both distal and proximal to an injured segment of the axon (4). In addition, somatopetal transfer of protein tracers, such as transfer of HRP from the uptake at the axonal terminals to the nerve cell bodies, has shown that even in intact axons in vivo a transport in the somatopetal direction occurs (7). Recent observations pertinent to this transport in the distal-proximal direction are reviewed in this section.

Compartments for Axonal Transport

In neurites in tissue culture, somatopetal movements of elongated sausage-shaped particles, considered to represent mitochondria and large numbers of spherical particles, have been observed. Similar particles moving in the distal-proximal direction have been observed in axons from excised mammalian sciatic nerves. The number of the relatively rapidly moving spherical particles in the somatopetal direction appears to exceed that in the somatofugal direction at a nearly 2:1 ratio (58) or more (56, 59). At the tips of the growing neurites, pinocytotic vesicles are formed, which ascend the axons as rounded organelles (53, 54, 55). Conceivably, some of the moving spherical particles represent such pinocytotic vesicles.

That materials transported in the somatopetal direction are confined to compartments in the axoplasm is evident from studies on retrograde transport of HRP. At the ultrastructural level, HRP is localized to vacuoles, cup-shaped bodies, occasional multivesicular bodies, and cisternal or tubular structures in the axoplasm (8, 14, 60). This agrees well with our belief that the neuronal cytoplasm is compartmentalized; channels as rough endoplasmic reticulum, Golgi apparatus, and smooth endoplasmic reticulum (SER) are isolated from the neuronal cytoplasm by a system of intracellular membranes (1). In the axon the SER forms a continuous channel system throughout the whole length of the axon (1). Recent studies have also shown that radiolabeled proteins are transported rapidly in the somatofugal direction within this system, reaching the terminals, where synaptic vesicles bud off from the network of the SER. The SER is a flexible system with movement of the channel walls as well as the included materials. Whether this SER provides a compartment for somatopetal transport in axons is not known, but occurrence of retrogradely transported HRP within cisternal structures has led Droz (1) to suggest that, while some channels of the SER are involved in rapid somatofugal transport, others may be involved in transport of materials in the somatopetal direction. However, it has been pointed out that some HRP-containing tubules in axons are fairly short, and others are actually elongated multivesicular bodies or dense bodies. It is therefore questionable whether many of the tubular structures involved in retrograde transport do represent SER (61).

Data on possible retrograde transport of transmitters are in conflict. While norepinephrine-specific fluorescence accumulates distal to a nerve crush (62), no dense-cored vesicles, which are presumptive storage sites for norepinephrine, are seen distal to a ligature (63). Recently, dopamine-β-hydroxylase (DBH), which is a marker for norepinephrine storage vesicles, has been found to be transported in the retrograde direction with the use of a stopflow technique (64). In this system, the rabbit sciatic nerve is incubated in chambers, which expose different regions of the nerve to different temperatures, and DBH accumulates distal to a cooled region. To explain the lack of large dense-cored vesicles, which accumulate only proximally to ligatures, it was suggested that DBH subjected to rapid retrograde transport is bound to empty catecholamine storage granules or fragments of their membranes.

Therefore, materials transported in the retrograde directions appear to consist of mitochondria and rapidly moving substances enclosed in organelles such as pinocytotic vesicles, lysosomal organelles, transmitter-containing vesicles, and SER. It is not known whether a somatopetal transport of substances dissolved in the axoplasm exists that is comparable to the slow axoplasmic transport.

Velocity of Retrograde Axonal Transport

In the anterograde or somatofugal direction there appear to be at least two different components of axonal transport. One of these has a slow velocity (in the order of 1 mm per day) and is called *slow transport,* while the other is faster and is called *fast transport.* The calculated value for the rate of the latter component varies in different experimental models. In mammalian and avian optic system a rate of 150 to 250 mm per day and in the goldfish a rate of 70 to 100 mm per day have been given (2). Ochs (3) found a relatively constant velocity of 410 mm per day for the fast axonal transport in the sciatic nerve in a variety of mammalian species. The rate of the retrograde or somatopetal transport also differs with different observations. Lubińska (4) estimated the retrograde transport of acetylcholinesterase to be about 125 mm per day or half the rate of transport in the anterograde direction. Ochs (3), on the other hand, calculated that the velocity for retrograde transport was about 200 mm per day. In frog sciatic nerves in vitro the retrograde transport of ^{3}H-leucine-labeled proteins is estimated at 60 mm per day or half of the forward rate (65). In contrast to these estimations of the rate of retrograde transport as half of the anterograde transport, the velocities of organelle movements in excised nerve fibers or neurites in tissue culture have been the same in both directions (58).

The velocity of transport of HRP appears to be in the order of 3–5 mm/hr (8, 66) and retrograde virus transport has been estimated to occur at 2.4 mm/hr for poliomyelitis (67) or at least 1.7 mm/hr for pseudorabies in mice (68). Both in rats and mice the rate of retrograde transport of nerve growth factor from the anterior eye chamber to the superior cervical ganglion amounts to about 2.5 mm/hr. In contrast, the rate of retrograde transport in sensory fibers in adult rats was 13 mm/hr. This may imply that different substances or organelles are transported with different rates in different nerve fiber systems (30).

Mechanisms for Retrograde Axonal Transport

The driving force for the retrograde transport of organelles or substances enclosed in organelles has not been defined. The transport is temperature-dependent; cooling of the nerve to 2°C arrests the flow (64). Both antero- and retrograde transport in frog sciatic nerves in vitro are inhibited by sodium cyanide, 2,4-dinitrophenol, and iodoacetic acid, which suggests that they are dependent on similar energy-producing processes (65). Microtubules have repeatedly been ascribed a significant role for fast axonal transport in the anterograde direction. A microfilament sliding hypothesis has been devised, whereby organelles are bound to microtubules by microfilaments and then transported (3). Mitochondria are often attached to microtubules ultrastructurally (69). Alternatively, sharing forces may provide zones of low viscositis around microtubules facilitating transport (70). It is not known whether microtubules are of importance for retrograde transport as well, but interference with microtubules by mitotic inhibitors as colchicine or vinblastine inhibits the transport (71). However, these drugs may also interfere with other structures of the axon, which makes it still premature to link retrograde transport directly to microtubules.

FATE OF EXOGENOUS MACROMOLECULES IN THE NERVE CELL BODY

In the nerve cell body HRP accumulates in vesicles of various sizes. Most of the vesicles in the perikaryon are larger than those in the axons indicating that the HRP-containing vesicles have coalesced with others (8). Structures suggestive of coalescing organelles are also abundant outside of the Golgi apparatus, while the Golgi sacs and vacuoles themselves are free of HRP product. Probably, therefore, the pinocytotic vesicles in neurons, as in other cells, fuse with primary lysosomes, giving rise to secondary lysosomes, in which protein breakdown may occur (72). Little is yet known concerning whether similar factors are of importance for the rate of protein breakdown in neurons as in other cells, i.e. concentration of the protein, pH in the lysosomes, absence or presence of inhibitors (73). In our experiments we have found that HRP disappears from the nerve cell bodies within 3–4 days, which may represent a breakdown or inactivation of the enzyme in the lysosomal organelles.

Apparently, certain biologically active substances such as hormones, viral nucleic acids, and hypothetical information carrying molecules should not be degraded by the hydrolytic lysosomal enzymes. For instance, the nucleus must be reached by nucleic acids from viruses, which replicate in this structure, but the pathway through the neuronal cytoplasm is not known. We have found HRP in vacuoles and tubules close to the outer nuclear membrane in neurons, but we were unable to trace it to the perinuclear space (50).

In recent years attention has been focused on transport phenomena in dendrites, where for instance acetylcholinesterase can be transported in smooth endoplasmic reticulum and appear at the dendritic surface especially near synapses (74). HRP also occurs within vesicles and the tubular structure in dendrites. In this context it

is interesting to note that tetanus toxin can pass from the motor nerve cell bodies to the presynaptic terminals where it exerts its action (75, 76). The transsynaptic passage of macromolecules may be a selective process because tetanus toxin passes in the adrenergic ganglia, while nerve growth factor does not (77).

The possibility also exists that some HRP, which has reached the nerve cell body, is transported back out into the axons. For instance, HRP injected into the superior cervical ganglion of the rabbit is incorporated into the nerve cell bodies and subsequently appears in the axons and in their terminal part in the iris (78).

In conclusion, macromolecules taken up by neurons may be broken down by the lysosomal system, transferred to the nucleus to influence the nerve cell body metabolism, transported into the dendrites and pass to the presynaptic terminals and, possibly, also be transported out into the axon again. Of great interest in neuropathology is to what extent heavy metals, which are not readily excreted from other cell types, can be taken up and accumulate for long periods of time in neurons.

IMPLICATIONS OF RETROGRADE AXONAL TRANSPORT

Growth and Regeneration of Neurons

For its development and maintenance, the nerve cell is greatly influenced by its field of innervation. For instance, experimentally induced destruction or atrophy of skeletal muscles causes hypoplasia of the ventral horns in the spinal cord (79). The nature of the signaling mechanism for such interactions is unknown, but it has been suggested that retrograde transport of macromolecules is involved.

In this respect a series of interesting observations has been made by Thoenen and co-workers on the biological importance of retrograde axonal transport of nerve growth factor (NGF) in adrenergic neurons. This factor promotes the growth of the peripheral sympathetic nervous system in newborn animals. It also causes a selective induction of tyrosine hydroxylase and dopamine-β-hydroxylase, which are key enzymes in the synthesis of norepinephrine. After unilateral injection of NGF into the anterior eye chamber of 8–10-day-old mice and rats, NGF can be detected in nerve cell bodies in the ipsilateral superior cervical ganglia, followed by an increase in tyrosine hydroxylase activity. It was therefore suggested that retrograde transport NGF, which can be synthesized locally, could represent a means for transfer of trophic information from the effector cells to the perikaryon of the innervating neuron (80).

Also the mature neuron needs information from the periphery, which is evident after an axonal injury (81), when the neuron can react with chromatolysis (82). Immediately after a nerve crush there is a transient diffusion of HRP into the injured axon proximal to the crush. At higher levels HRP is included in organelles, which are transported to the perikaryon, preceding the earliest morphological or biochemical alterations of the nerve cell bodies (50). By crushing the sciatic nerve in groups of rats at different levels, the rates for the ascent of the signal of chromatolysis and the axonal transport of HRP in injured axons could be compared. These were within the same range of 3–5 mm/hr, indicating a time relation between the two phenomena. Whether there is a causal relation is unknown, but two possibilities have been

suggested. First, the abruption of the normal transport of organelles coming from the periphery may carry the information to start the nerve cell body response to the injury. Second, some material (a "wound substance") from the injured axon or its surroundings may trigger the response (4).

In a series of experiments, we are studying the course of events of uptake and accumulation of HRP in regenerating nerve cell bodies. The facial nerves of mice are crushed followed by intravenous injection of HRP at varying time points. In these experiments, uptake and retrograde transport of HRP reappear when the regenerating axons have reached the end-plates and started to function, i.e. when the mice begin to move the vibrissae again. It may therefore be suggested that retrograde transport is involved not only in signaling the nerve cell's initial response after an axon injury, but also in informing the cell body that the axon terminals have reached their target area.

The retrograde transport of macromolecules from the synapses to the nerve cell bodies may have a further biological meaning for the mature neuron. As reviewed in a preceding section the uptake is dependent on the degree of synaptic activity. If such alterations in uptake also are reflected in alterations in transport, the nerve cell body may constantly be informed on the functional state of the synapses. In this way the retrograde transport may act as a feedback mechanism in the neuron. We are presently engaged in experiments trying to better define whether in vivo the neuronal tracer accumulation varies with the synaptic activity. For the advancement of this hypothesis a quantitative cytochemical technique is being developed, by which the amount of protein uptake in single isolated neurons can be determined.

Toxicological and Pharmacological Implications of Retrograde Transport

Of the various neurotoxins, the most extensive studies have been performed on the neural spread of tetanus toxin. As stated in previous sections, there are several recent studies showing that this toxin is taken up at the periphery of the axons and transported retrogradely to the nerve cell bodies. With the use of electron microscopic autoradiography, labeled toxin has also been localized over the presynaptic terminals of the motor neuron, where it exerts its action (75, 76).

It is not known whether other bacterial toxins can reach the motor neurons from the blood in this way. Since we have observed that Evans blue bound to serum albumin accumulates in motor neurons of the brain stem and spinal cord after intramuscular injection, the possibility must be evaluated that different drugs or chemicals bound to serum proteins may reach the lower motor neurons, and in this way bypass the so-called blood-brain barrier. Heavy metals might also bind to serum proteins, and the retrograde transport may therefore provide a potential hazard to the motor neurons exposing them to toxic metals such as lead, aluminum, and mercury. In fact, we have found that iron can be transported to motor neurons after intramuscular injection. However, very high doses of iron-dextran or ferritin, injected over several days, are needed to induce a histochemically detectable accumulation.

In pharmacology the retrograde axonal transport may have two implications. First, the potential risk that drugs may reach motor neurons after intravenous or

intramuscular injections should be evaluated. Further, the treatment of dermatological disorders may involve a risk in that substances are taken up by sensory neurons following their application to injured skin. Second, the possibility exists that drugs can be selectively introduced into diseased neurons, for instance, in conditions of intoxications or virus infections of the peripheral neuron. It might therefore be possible to find suitable carrier substances, by which chelating or antiviral agents may reach the nerve cell bodies. This might be an ideal treatment for recurrent herpes infection of the lips or conjunctiva, where the herpes virus probably remains latent in sensory ganglia neurons between the eruptions.

CONCLUDING REMARKS

The observation that neurons both in the central, peripheral, and autonomic nervous system can take up certain macromolecules at their axon terminals and transport them to the nerve cell bodies along the axons opens up several lines for future research in pharmacology and toxicology. Toxic agents, such as heavy metals, chemicals, and viruses, may reach the neurons by this mechanism to produce their effects. The specificity in the uptake should be evaluated for different agents, and the possibility of selectively interfering pharmacologically with the metabolism of a defined group of nerve cells should be explored. Probably, a specificity in macromolecular uptake might be obtained by the inclusion of pharmacological substances in carrier molecules.

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Literature Cited

1. Droz, B. 1975. Synthetic machinery and axoplasmic transport: Maintenance of neuronal connectivity. In *The Nervous System*, ed. D. B. Tower, Vol. 1, *The Basic Neurosciences*, ed. R. O. Brody, pp. 111–27. New York: Raven
2. Grafstein, B. 1975. Principles of anterograde transport in relation to studies of neuronal connectivity. In *The Use of Axonal Transport for Studies of Neuronal Connectivity*, ed. W. M. Cowan, M. Cuénod, pp. 47–67. Amsterdam: Elsevier
3. Ochs, S. 1974. Systems of material transport in nerve fibers (axoplasmic transport) related to nerve function and trophic control. *Ann. NY Acad. Sci.* 228:202–23
4. Lubińska, L. 1975. On axoplasmic flow. *Int. Rev. Neurobiol.* 17:241–96
5. Kristensson, K. 1970. Transport of fluorescent protein tracer in peripheral nerves. *Acta Neuropathol.* 16:293–300
6. Ellison, J. P., Clark, G. M. 1975. Retrograde axonal transport of horseradish peroxidase in peripheral autonomic nerves. *J. Comp. Neurol.* 161:103–14
7. Kristensson, K. 1975. Retrograde axonal transport of protein tracers. See Ref. 2, pp. 69–82
8. LaVail, J. H., LaVail, M. M. 1974. The retrograde intraaxonal transport of horseradish peroxidase in the chick visual system: A light and electron microscopic study. *J. Comp. Neurol.* 157: 303–58
9. LaVail, J. H. 1975. The retrograde transport method. *Fed. Proc.* 34: 1618–24
10. Walberg, F., Brodal, A., Hoddevik, G. H. 1976. A note on the method of retrograde transport of horseradish peroxidase as a tool in studies of afferent cerebellar connections, particularly those

from the inferior olive; with comments on the orthograde transport in Purkinje cell axons. *Exp. Brain Res.* 24:383–401
11. Klatzo, I. 1977. Cerebral oedema and ischemia. *Rec. Adv. Neuropathol.* In press
12. Olsson, Y. 1975. Vascular permeability in the peripheral nervous system. In *Peripheral Neuropathy;* ed. P. J. Dyck, P. K. Thomas, H. Lambert, pp. 190–200. Philadelphia: Saunders
13. Zachs, S. I., Saito, A. 1969. Uptake of exogenous horseradish peroxidase by coated vesicles in mouse neuromuscular junctions. *J. Histochem. Cytochem.* 17:161–70
14. Kristensson, K. 1977. Retrograde axonal transport of horseradish peroxidase. Uptake at mouse neuromuscular junction following systemic injection. *Acta Neuropathol.* 38:143–47
15. Stoeckel, K., Thoenen, H. 1975. Retrograde axonal transport of nerve growth factor: Specificity and biological importance. *Brain Res.* 85:337–41
16. Ryser, H. J.-P., Gabathuler, M.-P., Roberts, A. B. 1971. Uptake of macromolecules at the cell surfaces. *Biomembranes* 2:197–209
17. Jirmanová, I., Libelius, R., Lundquist, I., Thesleff, S. 1977. Protamine induced intracellular uptake of horseradish peroxidase and vacuolation in mouse skeletal muscle in vitro. *Cell Tiss. Res.* 176:463–73
18. Rambourg, A., Neutra, M., Leblond, C. P. 1966. Presence of a cell coat rich in carbohydrate at the surface of the cells in the rat. *Anat. Rec.* 154:41–72
19. Roseman, S. 1974. The biosynthesis of complex carbohydrates and their potential role in intercellular adhesion. In *The Cell Surface in Development,* ed. A. A. Moscana, pp. 255–71. New York: Wiley
20. Pappas, G. D. 1975. Ultrastructural basis of synaptic transmission. See Ref. 1, pp. 19–30
21. Bunt, A. H., Haschke, R. H., Lund, R. D., Calkins, D. F. 1976. Factors affecting retrograde axonal transport of horseradish peroxidase in the visual system. *Brain Res.* 102:152–55
22. Bernhard, W., Avrameas, S. 1971. Ultrastructural visualization of cellular carbohydrate components by means of concanavalin A. *Exp. Cell Res.* 64:232–36
23. Graham, R. C., Limpert, S., Kellermeyer, R. W. 1969. The uptake and transport of exogenous proteins in mouse liver. *Lab. Invest.* 20:298–304
24. Streit, P., Reubi, J. C. 1977. A new and sensitive staining method for axonally transported horseradish peroxidase (HRP) in the pigeon visual system. *Brain Res.* 126:530–37
25. Gurd, J. W. 1977. Identification of lectin receptors associated with rat brain postsynaptic densities. *Brain Res.* 126: 154–59
26. McLaughlin, B. J., Wood, J. G. 1977. The localization of Concanavalin A binding sites during photoreceptor synaptogenesis in the chick retina. *Brain Res.* 119:57–71
27. Koda, L. Y., Partlow, L. M. 1976. Membrane marker movement on sympathetic axons in tissue culture. *J. Neurobiol.* 7:157–72
28. Gonatas, N. K., Kim, S. U., Stieber, A., Avrameas, S. 1977. Internalization of lectins in neuronal gerl. *J. Cell Biol.* 73:1–13
29. Teichberg, S., Holtzman, E., Crain, S. M., Peterson, E. R. 1975. Circulation and turnover of synaptic vesicle membrane in cultured fetal mammalian spinal cord neurons. *J. Cell Biol.* 67: 215–30
30. Stoeckel, K., Schwab, M., Thoenen, H. 1975. Specificity of retrograde transport of nerve growth factor (NGF) in sensory neurons: A biochemical and morphological study. *Brain Res.* 89:1–14
31. Fillenz, M., Gagnon, C., Stoeckel, K., Thoenen, H. 1976. Selective uptake and retrograde axonal transport of dopamine-β-hydroxylase antibodies in peripheral adrenergic neurons. *Brain Res.* 114:293–303
32. Kahn, C. R. 1976. Membrane receptors for hormones and neurotransmitters. *J. Cell Biol.* 70:261–86
33. Johnson, R. T., Mims, C. A. 1968. Pathogenesis of viral infections of the nervous system. *N. Engl. J. Med.* 278: 23–30
34. Hökfelt, T., Ljungdahl, Å 1975. Uptake mechanisms as a basis for the histochemical identification and tracing of transmitter-specific neuron populations. See Ref. 2, pp. 249–305
35. Iversen, L. L., Bloom, F. E. 1972. Studies of the uptake of ^{3}H-GABA and ^{3}H-glycine in slices and homogenates of rat brain and spinal cord by electron microscopic autoradiography. *Brain Res.* 41:131–43
36. Snyder, S. H. 1975. Amino acid neuro-

transmitters: Biochemical pharmacology. See Ref. 1, pp. 355–62
37. Hunt, S., Cuénod, M. 1975. *Axonal Transport of ^{3}H-Glycine.* Presented at Int. Meet. Int. Soc. Neurochem., 5th, Barcelona
38. Watson, W. E. 1968. Centripetal passage of labelled molecules along mammalian motor axons. *J. Physiol. London* 196:122–23P
39. Gilmore, S. A., Daniels, J. S. 1974. An autoradiographic study on the question of retrograde axoplasmic transport following the injection of ^{3}H-leucine into the tongue. *Am. J. Anat.* 139:141–45
40. Gregoriades, G. 1976. The carrier potential of liposomes in biology and medicine. *N. Engl. J. Med.* 295:704–10
41. Adams, D. H., Joyce, G., Richardson, V. J., Ryman, B. E., Wiśniewski, H. M. 1977. Liposome toxicity in the mouse central nervous system. *J. Neurol. Sci.* 31:173–79
42. Andres, K. H. 1964. Mikropinozytose im Zentralnervensystem. *Z. Zellforsch. Mikrosk. Anat.* 64:232–36
43. Heuser, J. E., Reese, T. S. 1973. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. Cell Biol.* 57:315–44
44. Holtzman, E., Freeman, A. R., Kashner, L. A. 1971. Stimulation-dependent alterations in peroxidase uptake at lobster neuromuscular junctions. *Science* 173:733–36
45. Ripps, H., Shakib, M., MacDonald, E. D. 1976. Peroxidase uptake by photoreceptor terminals of the skate retina. *J. Cell Biol.* 70:86–96
46. Schacher, S., Holtzman, E., Hood, D. C. 1976. Synaptic activity of frog retinal photoreceptors. A peroxidase study. *J. Cell Biol.* 70:178–92
47. Stoeckart, R., Jansen, H. G., Kreike, A. J. 1974. Influence of castration on incorporation of exogenous peroxidase into "synaptic" vesicles of the median eminence. I. The basal part of the palisade zone in male rats. *Cell Tiss. Res.* 155:1–11
48. Litchy, W. J. 1973. Uptake and retrograde transport of horseradish peroxidase in frog sartorius nerve in vitro. *Brain Res.* 56:377–81
49. Singer, W., Holländer, H., Vanegas, H. 1977. Decreased peroxidase labeling of lateral geniculate neurons following deafferentation. *Brain Res.* 120:133–37
50. Kristensson, K., Olsson, Y. 1977. Retrograde transport of horseradish peroxidase in transected axons. 3. Entry into injured axons and subsequent localization in perikaryon. *Brain Res.* 126: 154–59
51. Westergaard, E., Brightman, M. W. 1973. Transport of proteins across normal cerebral arterioles. *J. Comp. Neurol.* 152:17–44
52. Broadwell, R. D., Brightman, M. W. 1976. Entry of peroxidase into neurons of the central and peripheral nervous systems from extracerebral and cerebral blood. *J. Comp. Neurol.* 166:257–84
53. Speidel, C. C. 1964. In vivo studies of myelinated nerve fibers. *Int. Rev. Cytol.* 16:173–231
54. Hughes, A. 1953. The growth of embryonic neurites. A study of chick neural tissues. *J. Anat.* 87:150–62
55. Pomerat, C. M., Hendelman, W. J., Raiborn, C. W., Massey, J. F. 1967. Dynamic activities of nervous tissue in vitro. In *The Neuron*, ed. H. Hydén, pp. 119–78. Amsterdam: Elsevier
56. Forman, D. S., Padjen, A., Siggins, G. R. 1975. *Movements of Optically Detectable Intra-Axonal Organelles.* Presented at Int. Meet. Int. Soc. Neurochem. 5th, Barcelona
57. Kirkpatrick, J. B., Bray, J. J., Palmer, S. M. 1972. Visualization of axoplasmic flow in vitro by Nomarski microscopy. Comparison to rapid flow of radioactive proteins. *Brain Res.* 43:1–10
58. Leestma, J. E. 1976. Velocity measurements of particulate neuroplasmic flow in organized CNS tissue cultures. *J. Neurobiol.* 7:173–83
59. Cooper, P. D., Smith, R. S. 1974. The movement of optically detectable organelles in myelinated axons of *Xenopus laevis*. *J. Physiol.* 242:77–97
60. Nauta, H. J. W., Kaiserman-Abramof, I. R., Lasek, R. J. 1975. Electron microscopic observations of horseradish peroxidase transported from the caudoputamen to the substantia nigra in the rat: Possible involvement of the agranular reticulum. *Brain Res.* 85: 373–84
61. Teichberg, S., Holtzman, E. 1973. Axonal agranular reticulum and synaptic vesicles in cultured embryonic chick sympathetic neurons. *J. Cell Biol.* 57: 88–108
62. Dahlström, A. 1965. Observations on the accumulation of noradrenalin in the proximal and distal parts of peripheral adrenergic nerves after compression. *J. Anat.* 99:677–89

63. Kappeler, K., Mayor, D. 1969. An electron microscopic study of the early changes distal to a constriction in sympathetic nerves. *Proc. R. Soc. London Ser. B* 172:53–63
64. Brimijoin, S., Helland, L. 1976. Rapid retrograde transport of dopamine-β-hydroxylase as examined by the stop-flow technique. *Brain Res.* 102:217–28
65. Edström, A., Hanson, M. 1973. Retrograde axonal transport of proteins in vitro in frog sciatic nerves. *Brain Res.* 61:311–20
66. Kristensson, K., Olsson, Y. 1976. Retrograde transport of horseradish peroxidase in transected axons. 2. Relations between rate of transfer from the injury to the perikaryon and onset of chromatolysis. *J. Neurocytol.* 4:653–61
67. Bodian, D., Howe, H. A. 1941. The rate of progression of poliomyelitis virus in nerves. *Bull. Johns Hopkins Hosp.* 69:79–85
68. Field, H. J., Hill, T. J. 1975. The pathogenesis of pseudorabies in mice: Virus replication at the inoculation site and axonal uptake. *J. Gen. Virol.* 26:145–48
69. Raine, C. S., Ghetti, B., Shelanski, M. L. 1971. On the association between microtubules and mitochondria within axons. *Brain Res.* 34:389–93
70. Gross, G. W. 1975. The microstream concept of neuronal transport. In *Physiology and Pathology of Dendrites, Adv. Neurol.* ed. G. W. Kreutzberg, 12:283–96. New York: Raven
71. Kristensson, K., Sjöstrand, J. 1972. Retrograde transport of protein tracers in the rabbit hypoglossal nerve during regeneration. *Brain Res.* 45:175–81
72. Holtzman, E., Teichberg, E., Abrahams, S. J., Citkowitz, E., Crain, S. M., Kawai, N., Peterson, E. R. 1973. Notes on the synaptic vesicles and related structures, endoplasmic reticulum, lysosomes and peroxisomes in nervous tissue and adrenal medulla. *J. Histochem. Cytochem.* 21:349–85
73. Gordon, A. H. 1973. The role of lysosomes in protein catabolism. In *Lysosomes in Biology and Pathology,* ed. J. T. Dingle, 3:89–137. Amsterdam: North-Holland & Am. Elsevier
74. Kreutzberg, G. W., Schubert, P. 1975. The cellular dynamics of intraneuronal transport. See Ref. 2, pp. 83–112
75. Price, D. L., Griffin, J. W., Peek, K. 1977. Tetanus toxin: Evidence for binding at presynaptic nerve endings. *Brain Res.* 121:379–84
76. Schwab, M. E., Thoenen, H. 1976. Electron microscopic evidence for a transsynaptic migration of tetanus toxin in spinal cord motor neurons: An electron microscopic and morphometric study. *Brain Res.* 105:213–27
77. Schwab, M. E., Thoenen, H. 1977. Selective transsynaptic migration of tetanus toxin after retrograde axonal transport in peripheral sympathetic nerves: A comparison with nerve growth factor. *Brain Res.* 122:459–74
78. Brownson, R. H., Uusitalo, R., Palkama, A. 1977. Intraaxonal transport of horseradish peroxidase in the sympathetic nervous system. *Brain Res.* 120:407–22
79. Price, D. L. 1974. The influence of the periphery on spinal motor neurons. *Ann. NY Acad. Sci.* 228:355–63
80. Paravicini, U., Stoeckel, K., Thoenen, H. 1975. Biological importance of retrograde axonal transport of nerve growth factor in adrenergic neurons. *Brain Res.* 84:279–91
81. Halperin, J. J., LaVail, J. H. 1975. A study of the dynamics of retrograde transport and accumulation of horseradish peroxidase in injured neurons. *Brain Res.* 100:253–69
82. Lieberman, A. R. 1974. Some factors affecting retrograde responses to axonal lesions. In *Essay on the Nervous System,* ed. R. Bellairs, G. G. Gray, pp. 71–105. Oxford: Clarendon