Agents and Actions
vol. 7/3 (1977)
Birkhäuser Verlag, Basel

Minireview

Retrograde Axonal and Transsynaptic Transport of Macromolecules: Physiological and Pathophysiological Importance[1]

by MARTIN E. SCHWAB and HANS THOENEN
Department of Pharmacology, Biozentrum, University of Basle, Klingelbergstrasse 70, CH-4056 Basle, Switzerland

Abstract

Anterograde and retrograde transport within axons and dendrites of nerve cells represent an integral part of the nerve cell function and biochemistry. A few exogenous macromolecules with most different molecular weights and physico-chemical properties (Nerve Growth Factor, tetanus toxin, cholera toxin, various lectins, antibodies against dopamine-β-hydroxylase) have been shown to be taken up and transported with the retrograde axonal transport in exceedingly high amounts if compared to most other macromolecules. Specific binding to membrane receptors seems to be the prerequisite for this highly efficient retrograde transport. Upon arrival at the cell body tetanus toxin is able to leave the neuron and to migrate transsynaptically to presynaptic nerve terminals of second-order neurons.

For NGF, tetanus toxin and some neurotropic viruses retrograde axonal transport eventually followed by transsynaptic transport may be crucially involved in their mechanism of action. Indirect evidence suggests the existence of a variety of endogenous molecules carrying specific information from the target cell and the nerve terminal to the cell body and eventually transsynaptically into second- or third-order neurons.

Transport phenomena within axons and dendrites of nerve cells have become an important and favorite topic in modern neurobiology. Two principal directions of transport can be distinguished: From the cell body to the peripheral parts of the neuron as anterograde or orthograde transport, or from the periphery to the cell body as retrograde transport. Two main transport velocities have been delineated for the anterograde transport in mammals and birds: A fast rate of about 400 mm/day and a slow rate of 1–20 mm/day [1–3]. Intermediary rates have been described for various specific enzymes or proteins [4–6]. The retrograde axonal transport ranges in the rate of the fast anterograde transport [7]. No slow retrograde axonal transport has been described to date.

Nerve cells are highly polarized structures, whose processes can extend over enormous distances within the body. The axon as the main and most often longest process of the neuron is devoid of ribosomes, i.e. all the soluble proteins of its cytoplasm and internal structures and organelles such as microtubules, microfilaments, neurofilaments and the components of the surface membrane have to be supplied by the cell body. The same is true for the machinery involved in neurotransmitter synthesis, release and inactivation. The considerable distances which often separate the nerve terminals from their cell bodies make an efficient transport system indispensable.

Anterograde transport

Biochemical analysis of the material transported with fast and slow anterograde transport has shown striking specificities with regard to the nature of the molecules transported with a particular speed [8, 9]. In general, soluble cytoplasmic proteins, e.g. tubulin, are transported at the slow rate, which therefore can be considered as a bulk flow of cytoplasm. In contrast, molecules transported with fast transport rates are either components of membraneous structures like vesicles and smooth endoplasmic reticulum or are associated with such organelles [10, 11]. They are essentially destined for the membranes of nerve terminals and synaptic vesicles, and also for the axolemma [12–16]. This association of fast transported material with vesicular and endoplasmic reticulum-like structures in the axon suggests a crucial involvement of these organelles in the transport mechanism [10, 1]. In spinal cord and cranial nerve motoneurons and in

[1] Dedicated to K. Bucher on the occasion of his 65th birthday.

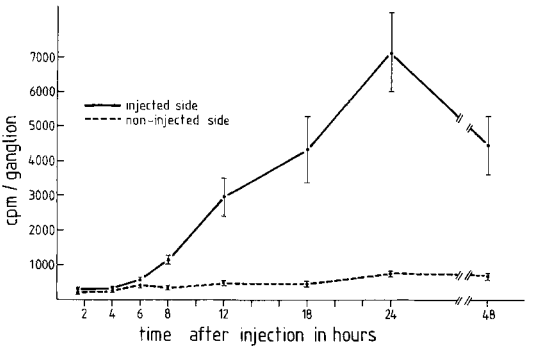


Figure 1
Time course of accumulation of radioactivity in superior cervical ganglia after unilateral injection of 25 μCi of ^{125}I-labeled antibodies to DBH into the anterior eye chamber. 1 to 48 h after injection the animals were killed, the ganglia removed and their radioactivity determined. The values given represent the mean ± S.E.M. of groups of 4–6 animals. (From FILLENZ et al. [29].)

adrenergic fibers a considerable part of the rapidly transported molecules return to the cell body by retrograde transport after a relatively short stay at the nerve terminal [17–20].

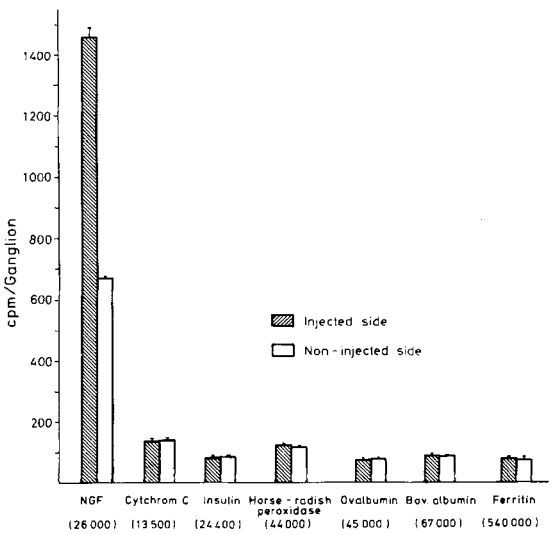


Figure 2
Comparison between the accumulation of radioactivity in superior cervical ganglia after unilateral intraocular injection of ^{125}I-labeled NGF and various other ^{125}I-labeled proteins. The isoelectric points of the proteins were: NGF 9.3; cytochrome c 9.8; insulin 5.4; horseradish peroxidase 9.7; ovalbumin 4.6; bovine serum albumin 4.8; ferritin 4.6. The molecular weights of the injected proteins are given in parentheses. In each case the injected radioactivity amounted to 1 μCi. The labeled proteins were injected 14 h before dissecting the ganglia. Each column represents the mean ± S.E.M. of 7–10 animals. (From STÖCKEL et al. [23].)

Uptake and retrograde transport of macromolecules

Two groups of macromolecules can be distinguished according to their efficiency of retrograde transport. For the majority of macromolecules, e.g. horseradish peroxidase, albumin, ferritin etc., a retrograde transport can only be detected if they are injected into the vicinity of nerve terminals in extremely high concentrations [21, 22]. In contrast, detectable retrograde transport in various parts of the peripheral and central nervous system occurs with much lower concentrations for a few particular macromolecules. So far, such a selective, highly efficient retrograde transport has been found for Nerve Growth Factor (NGF, MW 26'000) [7, 23, 24], tetanus toxin (MW 150'000) [7, 25, 26], a non-toxic fragment of the tetanus toxin molecule (MW 46'000), cholera toxin (MW 68'000) [27], the lectins wheat germ agglutinin (MW 34'000), ricin (MW 60'000) and phytohaemagglutinin (MW 110'000) [27], unpublished observations) and for antibodies against the enzyme dopamine-β-hydroxylase (MW 160'000) [28, 29] (Figs. 1, 2, 3, 4). The selectivity of retrograde axonal transport of these macromolecules seems to be due to the presence of specific binding sites for these molecules at the nerve terminal membrane. Indeed, the highly efficient retrograde transport of cholera toxin, which binds to GM_1 gangliosides, and of tetanus toxin, which binds to GD_{1b} and GT_1 di- and trisialogangliosides [30], could be blocked by simultaneous injection of the corresponding gangliosides [27]. On the other hand, the selective retrograde transport of NGF is confined to peripheral adrenergic and sensory neurons, but does not occur in mononeurons [7]. This finding agrees with the fact that high affinity surface receptors for NGF are present only on adrenergic and sensory ganglion cells. Oxidation of the tryptophan residues of the NGF molecule causes a gradual loss of the biological activity (as judged by fiber outgrowth from embryonic chicken dorsal root ganglia) and concommitantly the disappearance of retrograde transport [23]. Conversely, horseradish peroxidase, a molecule which is not transported in detectable amounts if injected alone in low concentrations, is transported retrogradely in high amounts if coupled to biologically active NGF [31]. For lectins binding to specific sugar residues of cell surface membrane glycoproteins or glycolipids is well documented [32].

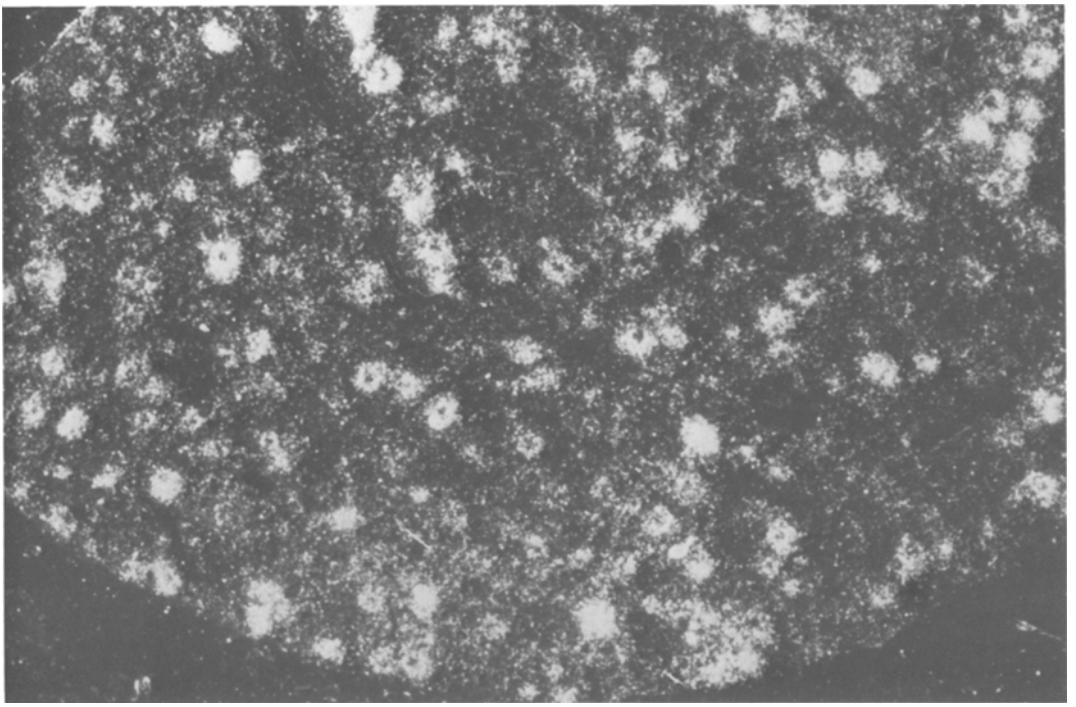


Figure 3
Ganglion cells of the rat superior cervical ganglion labeled by retrograde axonal transport of ^{125}I nerve growth factor. Rats were killed 14 h after injection of 10 μl (30 μCi) ^{125}I-NGF into the anterior eye chamber and 30 μl (90 μCi) into the submandibular gland. Light microscopic autoradiograph shows a cross-section of the superior cervical ganglion. (Magnification 80×.)

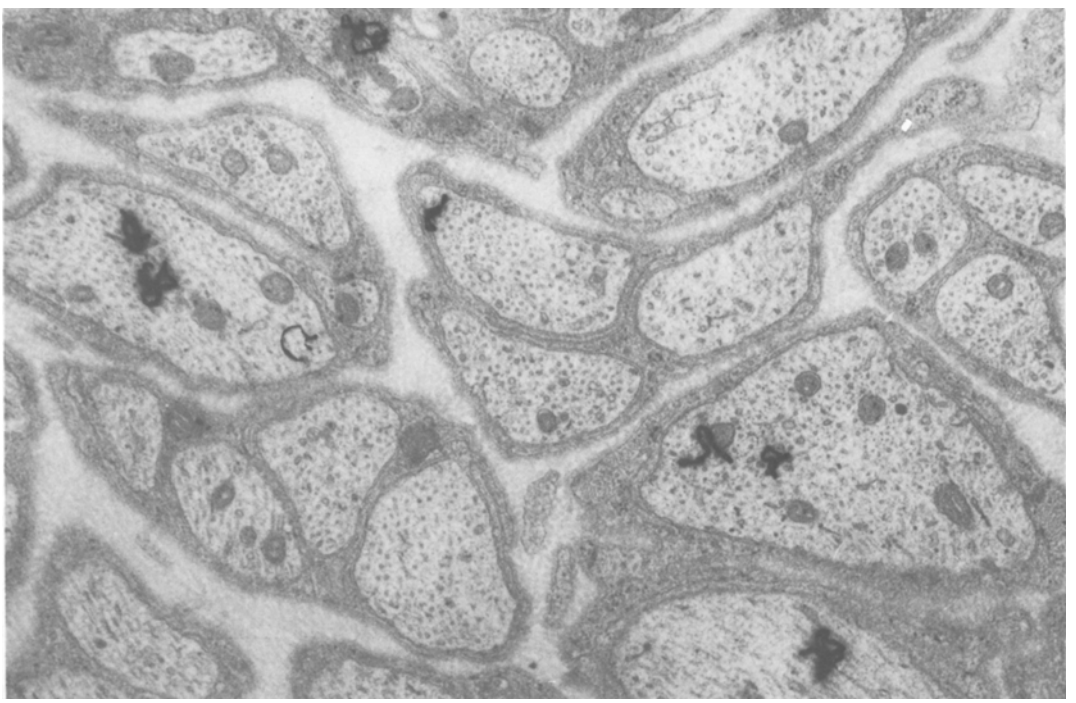
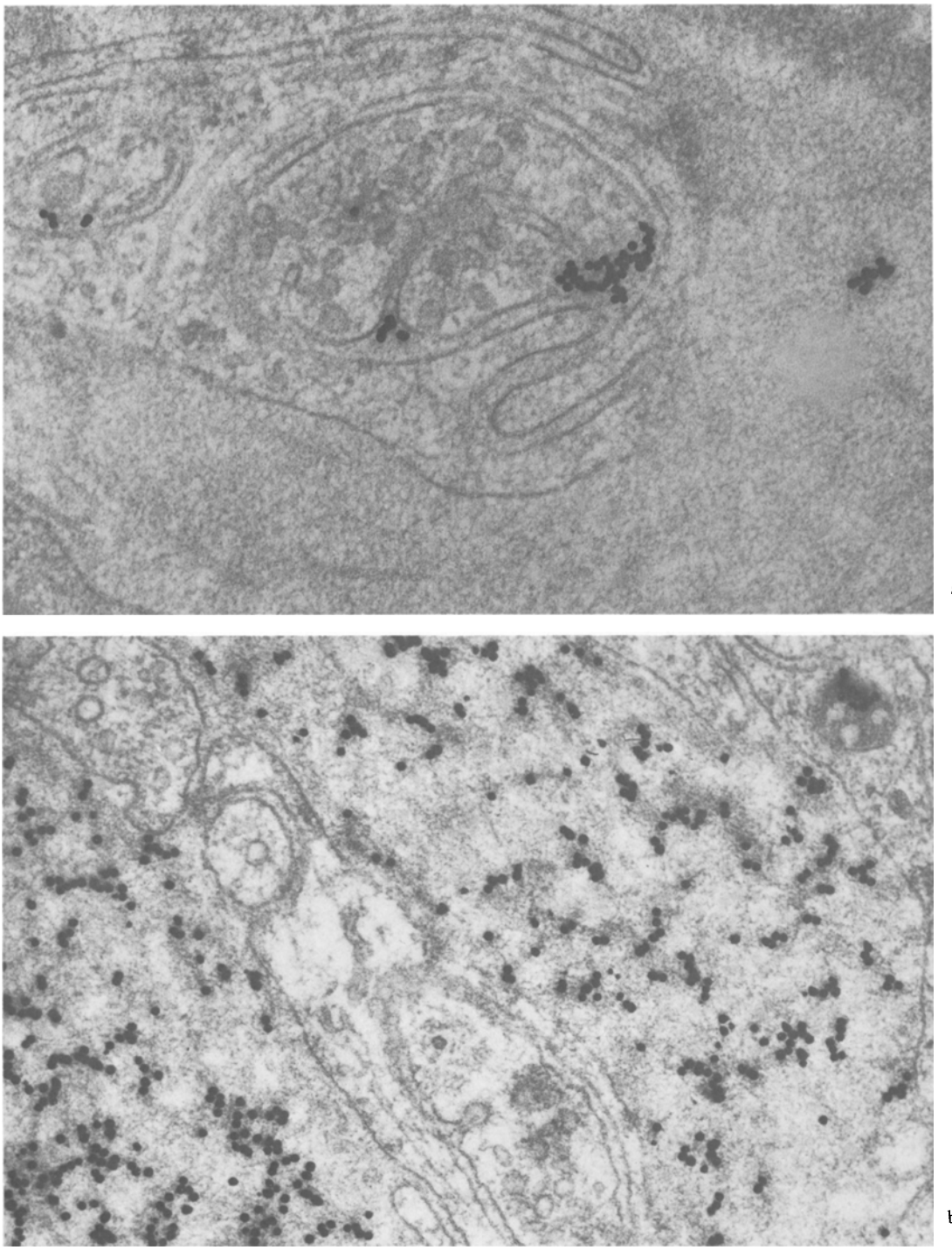


Figure 4
Labeled axons of ganglion cells of the rat superior cervical ganglion showing the intraaxonal localization of a lectin (wheat germ agglutinin) during retrograde transport. Rats were injected as indicated in Figure 3 and processed for electron microscopic autoradiography. (Magnification 11,000×.)



a.

b.

Figure 5
Rats were injected into the anterior eye chamber with 10 μl of colloidal gold coated with tetanus toxin or albumin and fixed 1 hr later. – (5a) Gold particles coated with *tetanus toxin* adhere to the membrane of nerve terminals in the rat iris. Arrow points to a particle inside a nerve terminal. (Magnification 100,000×.) – (5b) *No* association of gold particles with neuronal membranes and *no* uptake into nerve terminals can be seen with *albumin*-coated *gold* particles. (Magnification 75,000×.)

Morphological studies in the rat iris have shown a selective binding to nerve terminals followed by uptake and retrograde transport of tetanus toxin coupled to colloidal gold as an electron-dense tracer (Fig. 5a). In contrast, albumin-coated gold particles were neither associated with neuronal membranes nor taken up by nerve terminals [33] (Fig. 5b). Inside the axons, the tetanus toxin-gold complexes as well as the NGF-horseradish peroxidase coupling product [31] were located within smooth membrane cisternae, suggesting an important role of the smooth endoplasmic reticulum as a carrier for retrograde axonal transport.

All these data strongly speak in favor of specific binding sites as being the prerequisites for uptake and highly efficient retrograde transport.

Transsynaptic transport

Electron microscopic autoradiographic studies have been performed on rat sympathetic ganglia and spinal cord at the time of arrival of retrogradely transported ^{125}-I-labeled tetanus toxin [34, 35]. As expected, labeled cell bodies, axons and dendrites of adrenergic ganglion cells or spinal cord motoneurons were present. In addition radioactivity could be detected inside presynaptic terminals making contact with labeled neurons. As the labeling density over the glia was very low in comparison to the extremely high specific labeling of presynaptic terminals, and retrograde intraaxonal transport has been shown to be the only way by which tetanus toxin can reach the central nervous system, this phenomenon suggested a retrograde transsynaptic migration of tetanus toxin. Surprisingly, this retrograde transsynaptic migration following retrograde axonal transport was confined to tetanus toxin and did not occur after retrograde axonal transport of NGF. The mechanism of this selective transsynaptic migration of a macromolecule is still unclear.

Conclusions and possible physiological significances

Certain exogenous macromolecules bind with high affinity to components of the surface membrane of nerve terminals. This binding is followed by uptake and subsequent retrograde transport with a rate of several millimeters per hour. At the level of the cell body the transported material is redistributed: Part of it becomes incorporated into lysosomes, part stays in the smooth endoplasmic reticulum in the perikaryon or is transported into the dendrites [34, 35].

None of these macromolecules has been observed to enter the cell nucleus in detectable amounts. In adrenergic ganglion cells retrogradely transported NGF triggers a selective increase in the synthesis of tyrosine hydroxylase, the rate-limiting enzyme for the synthesis of the adrenergic transmitter noradrenaline [36, 37]. On the other hand, tetanus toxin is able to leave the neurons after its retrograde transport and is taken up again by presynaptic terminals of second-order neurons.

It may be reasonable to assume that this pathway of retrograde axonal transport, eventually followed by transsynaptic transfer, could have a most important function as a way of communication between the nerve terminal and its surrounding and the cell body as well as between individual neurons. Endogenous molecules could carry specific signals and information from the target organ to the regulatory machinery of the innervating neuron and even further up into second-order neurons. It is known from in vivo and tissue culture experiments that general 'trophic' influences as well as specific signals are produced by a target organ and act on innervating neurons [38, 39]. Many lines of evidence suggest that the signal for chromatolysis, i.e. the complex response of the cell body to lesion of its axon, travels by retrograde transport [40]. In adrenergic ganglion cells the interruption after axotomy of the steady supply of the neurons with NGF by retrograde transport has been suggested to trigger the chromatolytic response and eventually cell death [41]. Detachment of synapses and retraction from the postsynaptic cells with concommitant failure of impulse transmission as a consequence of axotomy of the *postsynaptic* cell is well documented for sympathetic ganglion cells and spinal cord motoneurons. This pathologically important retrograde transsynaptic effect could be mediated by a message which travels retrogradely across the synapse in the same way as tetanus toxin [42–46]. In various neuron systems of the CNS retrograde and anterograde transneuronal degenerations are well-known phenomena [47].

Clinical aspects and possible therapeutic consequences

The transsynaptic migration of tetanus toxin following its retrograde axonal transport finally answers the question of how the toxin, which

cannot penetrate the blood barrier, reaches its site of action in the CNS, i.e. the inhibitory nerve terminals ending on spinal cord motoneurons. Electrophysiological and biochemical studies have shown earlier that tetanus toxin acts by selectively blocking the release of the inhibitory transmitters glycine and GABA [48–50].

In addition to tetanus toxin various neurotropic viruses have been shown to reach their targets by retrograde axonal transport (herpes, rabies, pseudorabies, possibly poliomyelitis) [51–56]. As is the case for a generalized as opposed to a local tetanus [7], distribution via the blood stream throughout the body (vivemia in the case of a virus) would bring the toxin or virus in contact with nerve terminals in the entire periphery of the body wherefrom retrograde transport to the CNS or to autonomic and sensory ganglia can occur. It will be of high importance for the understanding of the mechanism of infection of many neurotropic viruses to study carefully their ability to penetrate the blood-brain barrier, their way of interaction with the nerve cell membrane and their possible retrograde and transsynaptic transport.

In the course of the study of the tetanus toxin molecule, a non-toxic fragment of molecular weight 46'000 has been prepared by BIZZINI et al. [57] at the Pasteur Institute in Paris. In spite of the loss of toxicity, the ability to bind to gangliosides is fully preserved as is its retrograde transport. The use of such a molecule as a carrier could make it possible to bring drugs into cells which normally are not taken up. In the case of horseradish peroxidase coupled to NGF this assumption has been shown to be fully valid [31]. Additional important aspects are the selectivity of tetanus toxin for binding sites which are enriched on neuronal membranes. Thus, using the tetanus toxin fragment would mean the construction of a specific neurotropic carrier. Furthermore, the carrier-drug complex will most probably use the same intracellular compartment for retrograde transport as the transported virus, which gives the drug a unique chance to reach its target in a most direct way. Studies for the construction and use of such a neurotropic carrier for drugs are under way in the Pasteur Institute at present.

Received 4 July 1977.

References

[1] S. OCHS, *Systems of Material Transport in Nerve Fibers (Axoplasmic Transport) Related to Nerve Function and Trophic Control*, Ann. N.Y. Acad. Sci. *228*, 202–223 (1974).

[2] J.W. GRIFFIN, D.L. PRICE, D.B. DRACHMAN and W.K. ENGEL, *Axonal Transport to and from the Motor Nerve Ending*, Ann. N.Y. Acad. Sci. *274*, 31–45 (1976).

[3] P. SCHUBERT, Y.W. KREUTZBERG and H.O. LUX, *Neuroplasmic Transport in Dendrites: Effect of Colchicine on Morphology and Physiology of Motoneurons in the Cat*, Brain Res. *47*, 331–343 (1972).

[4] M. WILLIARD, W.M. COWAN and P.R. VAGELOS, *The Polypeptide Composition of Intra-Axonally Transported Proteins: Evidence for Four Transport Velocities*, Proc. natl. Acad. Sci. 2183–2187 (1974).

[5] L. LUBINSKA and S. NIEMIERKO, *Velocity and Intensity of Bidirectional Migration of AchE in Transsected Nerves*, Brain Res. *27*, 329–342 (1971).

[6] H. THOENEN, U. OTTEN and F. OESCH, *Axoplasmic Transport of Enzymes Involved in the Synthesis of Noradrenaline: Relationship Between the Rate of the Transport and Subcellular Distribution*, Brain Res. *62*, 471–475 (1973).

[7] K. STÖCKEL, M. SCHWAB and H. THOENEN, *Comparison Between the Retrograde Axonal Transport of Nerve Growth Factor and Tetanus Toxin in Motor, Sensory and Adrenergic Neurons*, Brain Res. *99*, 1–16 (1975).

[8] J.L. BARKER, J.H. NEALE and H. GAINER, *Rapidly Transported Proteins in Sensory, Motor and Sympathetic Nerves of the Isolated Frog Nervous System*, Brain Res. *105*, 497–515 (1976).

[9] J.O. KARLSSON, *Proteins of Axonal Transport: Investigation of Solubility Characteristics and Behaviour in gel Filtration*, J. Neurochem. *27*, 1135–1143 (1976).

[10] B. DROZ, A. RAMBOURG and H.L. KOENIG, *The Smooth Endoplasmic Reticulum: Structure and Role in the Renewal of Axonal Membrane and Synaptic Vesicles by Fast Axonal Transport*, Brain Res. *93*, 1–13 (1975).

[11] J. SCHONBACH, CH. SCHONBACH and M. CUÉNOD, *Rapid Phase of Axoplasmic Flow and Synaptic Proteins: An Electron Microscopical Autoradiographic Study*, J. Comp. Neurol. *141*, 485–498 (1971).

[12] B. DROZ, H. KOENIG and L. DI GIAMBERARDINO, *Axonal Migration of Protein and Glycoproteins to Nerve Endings: I. Radioautographic Analysis of Renewal of Protein in Nerve Endings of Chicken Ciliary Ganglion after Intracerebral Injection of H_3-lysine*, Brain Res. *60*, 93–127 (1973).

[13] G. BENNETT, L. DI GIAMBERARDINO, H.L. KOENIG and B. DROZ, *Axonal Migration of Protein and Glycoprotein to Nerve Endings. II. Radioautographic Analysis of the Renewal of Glycoproteins in Nerve Endings of Chicken Ciliary Ganglion after Intracerebral Injection of H_3-glucosamine*, Brain Res. *60*, 129–146 (1973).

[14] L. DI GIAMBERARDINO, G. BENNETT, H.L. KOENIG and B. DROZ, *Axonal Migration of Protein and Glycoprotein to Nerve Endings. III. Cell Fraction Analysis of Chicken Ciliary Ganglion after Intracerebral Injection of Labeled Precursors, of Proteins and Glycoproteins*, Brain Res. *60*, 147–159 (1973).

[15] P. CANCALON and L.M. BEIDLER, *Differences in the Composition of the Polypeptides Deposited in the Axon and the Nerve Terminals by Fast Axonal Trans-*

port in the Garfish Olfactory Nerve, Brain Res. *121*, 215–227 (1977).

[16] V. KRYGIER-BREVART, D.G. WEISS, E. MEHL, P. SCHUBERT and G.W. KREUTZBERG, *Maintenance of Synaptic Membranes by the Fast Axonal Flow*, Brain Res. *77*, 97–110 (1974).

[17] M.A. BISBY, *Orthograde and Retrograde Axonal Transport of Labeled Protein in Motoneurons*, Exp. Neurol. *50*, 628–640 (1976).

[18] M. FRIZELL, W.G. MCLEAN J. SJÖSTRAND, *Retrograde Axonal Transport of Rapidly Migrating Labelled Proteins and Glycoproteins in Regenerating Peripheral Nerves*, J. Neurochem. *27*, 191–196 (1976).

[19] I. NAGATSU, Y. KONDO, T. KATO and T. NAGATSU, *Retrograde Axoplasmic Transport of Inactive Dopamine-β-hydroxylase in Sciatic Nerves*, Brain Res. *116*, 277–285 (1976).

[20] S. BRIMIJOIN and L. HELLAND, *Rapid Retrograde Transport of Dopamine-β-hydroxylase as Examined by the Stop-Flow Technique*, Brain Res. *102*, 217–228 (1976).

[21] K. KRISTENSSON, Y. OLSSON and J. SJOSTRAND, *Axonal Uptake and Retrograde Transport of Exogenous Protein in the Hypoglossal Nerve*, Brain Res. *32*, 399–406 (1971).

[22] H.J.W. NAUTA, M.B. PRITZ and R.J. LASEK, *Afferents to the Rat Caudoputamen Studied with Horseradish Peroxidase. An Evaluation of a Retrograde Neuroanatomical Research Method*, Brain Res. *67*, 219–238 (1974).

[23] K. STÖCKEL, U. PARAVICINI and H. THOENEN, *Specificity of the Retrograde Axonal Transport of Nerve Growth Factor*, Brain Res. *76*, 413–421 (1974).

[24] K. STÖCKEL, M. SCHWAB and H. THOENEN, *Specificity of Retrograde Transport of Nerve Growth Factor (NGF) in Sensory Neurons: A Biochemical and Morphological Study*, Brain Res. *89*, 1–14 (975).

[25] G. ERDMANN, H. WIEGAND and H.H. WELLHÖNER, *Intraaxonal and Extraaxonal Transport of ^{125}I-Tetanus Toxin in Early Local Tetanus*, Naunyn-Schmiedebergs Arch. Pharm. *290*, 357–373 (1975).

[26] D.L. PRICE, J. GRIFFIN, A. YOUNG, K. PECK, and A. STOCKS, *Tetanus Toxin: Direct Evidence for Retrograde Intraaxonal Transport*, Science *188*, 945–947 (1975).

[27] K. STÖCKEL, M.E. SCHWAB, and H. THOENEN, *Role of Gangliosides in the Uptake and Retrograde Axonal Transport of Cholera and Tetanus Toxin as Compared to Nerve Growth Factor and Wheat Germ Agglutinin*, Brain Res. (in press).

[28] M.G. ZIEGLER, J.A. THOMAS and D.M. JACOBOWITZ, *Retrograde Axonal Transport of Antibody to Dopamine-β-hydroxylase*, Brain Res. *104*, 390–395 (1976).

[29] M. FILLENZ, C. GAGNON, K. STÖCKEL and H. THOENEN, *Selective Uptake and Retrograde Axonal Transport of Dopamine-β-hydroxylase Antibodies in Peripheral Adrenergic Neurons*, Brain Res. *114*, 293–303 (1976).

[30] W.E. VAN HEYNINGEN, *Gangliosides as Membrane Receptors for Tetanus Toxin, Cholera Toxin and Serotonin*, Nature *249*, 415–417 (1974).

[31] M.E. SCHWAB, *Ultrastructural Localization of a Nerve Growth Factor–Horseradish Peroxidase (NGF–HRP) Coupling Product after Retrograde Axonal Transport in Adrenergic Neurons*, Brain Res. *130*, 190–196 (1977).

[32] G.L. NICOLSON, *The Interactions of Lectins with Animal Cell Surfaces*, Intern. Rev. Cytol. *39*, 89–190 (1974).

[33] M.E. SCHWAB and H. THOENEN, *Selective Binding Uptake and Retrograde Transport of Tetanus Toxin by Nerve Terminals in the Rat Iris*, J. Cell. Biol. (submitted).

[34] M.E. SCHWAB and H. THOENEN, *Electron Microscopic Evidence for a Trans-synaptic Migration of Tetanus Toxin in Spinal Cord Motoneurons: An Autoradiographic and Morphometric Study*, Brain Res. *105*, 213–227 (1976).

[35] M.E. SCHWAB and H. THOENEN, *Selective Trans-Synaptic Migration of Tetanus Toxin after Retrograde Axonal Transport in Peripheral Sympathetic Nerves: A Comparison with Nerve Growth Factor*, Brain Res. *122*, 459–474 (1977).

[36] U. OTTEN, M. SCHWAB, C. GAGNON and H. THOENEN, *Selective Induction of Tyrosine Hydroxylase and Dopamine-β-hydroxylase by Nerve Growth Factor: Comparison Between Adrenal Medulla and Sympathetic Ganglia of Adult and Newborn Rats*, Brain Res. (in press).

[37] U. PARAVICINI, K. STÖCKEL and H. THOENEN, *Biological Importance of Retrograde Axonal Transport of Nerve Growth Factor in Adrenergic Neurons*, Brain Res. *84*, 279–291 (1975).

[38] K. FUXE, L. OLSON and Y. ZOTTERMAN, *Dynamics of Degeneration and Growth in Neurons* (Pergamon Press, Oxford, 1974).

[39] E. COOPER, J. DIAMOND and C. TURNER, *The Effects of Nerve Section and of Colchicine Treatment on the Density of Mechanosensory Nerve Endings in Salamander skin*, J. Physiol. *264*, 725–749 (1977).

[40] B.G. CRAGG, *What is the Signal for Chromatolysis?* Brain Res. *23*, 1–21 (1970).

[41] I.A. HENDRY and J. CAMPBELL, *Morphometric Analysis of Rat Superior Cervical Ganglion after Axotomy and Nerve Growth Factor Treatment*, J. Neurocytol. *5*, 351–360 (1976).

[42] M.R. MATTHEWS and V.H. NELSON, *Detachment of Structurally Intact Nerve Endings from Chromatolytic Neurones of Rat Superior Cervical Ganglion during the Depression of Synaptic Transmission Induced by Post-Ganglionic Axotomy*, J. Physiol. *245*, 91–135 (1975).

[43] D. PURVES, *Functional and Structural Changes in Mammalian Sympathetic Neurones Following Interruption of their Axons*, J. Physiol. *252*, 429–463 (1975).

[44] D. PURVES, *Functional and Structural Changes in Mammalian Sympathetic Neurones Following Colchicine Application to Postganglionic Nerves*, J. Physiol. *259*, 159–175 (1976).

[45] B.E.H. SUMNER, *An Ultrastructural Study of Normal and Injured Hypoglossal Nuclei after Injection of Horseradish Peroxidase*, Exp. Brain Res. *23*, 463–470 (1975).

[46] L. LANDMESSER and G. PILAR, *Fate of Ganglionic Synapses and Ganglion Cell Axons during Normal and Induced Cell Death*, J. Cell Biol. *68*, 357–374 (1976).

[47] W.M. COWAN, *Anterograde and Retrograde Transneuronal Degeneration in the Central and Peripheral Nervous System*, in: *Contemporary Research Methods in Neuroanatomy* (Eds. W.J.H. Nauta and S.O.E. Ebbesson; Springer, Berlin, 1970), pp. 217–251.

[48] D.R. Curtis and W.C. De Groat, *Tetanus Toxin and Spinal Inhibition*, Brain Res. *10*, 208–212 (1968).
[49] D.R. Curtis, D. Felix, C.J.A. Game and R.M. McCulloch, *Tetanus Toxin and the Synaptic Release of GABA*, Brain Res. *51*, 358–362 (1973).
[50] R.H. Osborne and H.F. Bradford, *Tetanus Toxin Inhibits Amino Acid Release from Nerve Endings in vitro*, Nature New Biol. *244*, 157–158 (1973).
[51] K. Blinzinger and A.P. Anzil, *Neural Route of Infection in Viral Diseases of the Central Nervous System*, Lancet *7*, 1374–1377 (1974).
[52] M. Dolivo (personal communication).
[53] K. Kristensson, B. Ghetti and H.M. Wisniewski, *Study of the Propagation of Herpes Simplex Virus (Type 2) into the Brain after Intraocular Injection*, Brain Res. *69*, 189–202 (1974).
[54] F.A. Murphy, S.P. Baner, A.K. Harrison and W.C. Winn, Jr., *Comparative Pathogenesis of Rabies and Rabies-like Viruses. Viral Infection and Transmit from Inoculation Site to the Central Nervous System;* Pathol. Invest. *28*, 361–376 (1973).
[55] R.W. Price, B.J. Kath and A.L. Notkins, *Latent Infection of the Peripheral ANS with Herpes Simplex Virus*, Nature *257*, 686–688 (1975).
[56] M.A. Walz, R.W. Price and A.L. Notkins, *Latent Ganglionic Infection with Herpes Simplex Virus Types 1 and 2: Viral Reactivation in vivo after Neurectomy*, Science *184*, 1185–1187 (1974).
[57] B. Bizzini, K. Stöckel and M. Schwab, *An Antigenic Polypeptide Fragment Isolated from Tetanus Toxin: Chemical Characterization, Binding to Gangliosides and Retrograde Axonal Transport in Various Neuron Systems*, J. Neurochem. *28*, 529–542 (1977).