Occasional review

A proposal for a classification of neuropathies according to their axonal transport abnormalities

JOHANNES JAKOBSEN, PER SIDENIUS, HANS BRÆNDGAARD

From the Diabetes Research Laboratory, Second University Clinic of Internal Medicine, and Department of Neurology, Aarhus Kommunehospital, Aarhus C, Denmark

SUMMARY Recent studies on axonal transport in experimental neuropathy are reviewed and the following combinations of pathological changes and underlying axonal transport abnormalities are proposed for a classification of polyneuropathies. Alterations of the anterograde transport of *slow component a (SCa)* leads to changes of the dimensions of the axon calibre without the occurrence either of overt neuropathy or fibre loss. Thus damming of SCa in β,β' -iminodipropionitrile (IDPN) intoxication results in axonal swelling in nerve roots whereas decrease of SCa leads to atrophy distal to the swellings in IDPN intoxication and in streptozotocin induced diabetes as well. Decrease in the amount of material conveyed within the *anterograde fast component (aFC)* leads to acute axonal degeneration including break down of axons and fibre loss. This state occurs in acute hypoglycaemia and in doxorubicin intoxication. The most frequent type of polyneuropathy, namely distal axonopathy with accumulation of axon organelles leading to distal fibre loss, is associated with decrease in amount of the *retrograde fast component (rFC)*. The transport is impaired before the appearance of symptoms and electrophysiological signs of neuropathy develop in the intoxications induced by parabromophenylacetylurea, acrylamide and 2·5 hexanedione, and the severity of neuropathy is proportional to the rFC impairment.

Although improvement in diagnosis of peripheral neuropathies has been achieved during recent years these conditions are still poorly distinguished. Diagnosis relies on symptoms, neurological signs, neurophysiological and chemical findings, and in some cases biopsy studies. Most classifications are based on histological criteria; the latest distinguishes between four categories of neuropathies, namely neuronopathies, proximal axonopathies, distal axonopathies, and myelinopathies.¹ These categories, however, are not aetiological but merely a description of pathological changes that result from a variety of causes. In fact, demyelination can occur secondary to axonal atrophy² and the same neurotoxic compound can produce more than one neuronal reaction depending on dosage.^{3,4}

It is not possible yet to group the neuropathies

according to biochemical criteria related to disease mechanisms, nor does this goal appear to be achievable in the near future. In the meantime, we propose a new classification based on axonal transport abnormalities combined with structural alterations. In our opinion, the inclusion of alterations in axon function provides new insight in the pathogenesis of these conditions.

The axonal transport system

Studies by Ochs *et al* during the sixties established the existence of the axonal transport system and clarified the dependence of the fast anterograde component on oxidative phosphorylation.^{5,6} Later the anterograde slow components and the retrograde fast component were described. Ultrastructural and gelelectrophoretic studies by the groups of Lasek and Willard have made it clear that each component of the transport system is identical with a specific axonal organelle (table 1).⁷⁻⁹ The pathological consequence of this structural hypothesis on axonal transport is that impairment of the various transport components

Address for reprint requests: Dr J Jakobsen, Department of Neurology, Aarhus Kommunehospital, DK-8000, Aarhus C, Denmark.

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Table 1 Axonal organelles, their corresponding components of the axonal transport system, and some of their physical and chemical characteristics

Axonal organelle	Transport component	Transport velocity	Molecular constituents
Neurofilaments	Slow component a (SCa) group V	1 mm/d	Triplet of neurofilament proteins (200, 145, and 68 kdalton)
Neurotubules	SCa/SCb	2 mm/d	α -tubulin, β -tubulin
Microfilament network	Slow component b (SCb) group IV	4 mm/d	Actin, calmodulin, fodrin, enzymes of intermediary metabolism etc.
Mitochondria	Group II	50 mm/d	Mitochondrial enzymes
Vesicles, smooth endoplasmic reticulum	Anterograde fast component (aFC), group I	400 mm/d	Glycoproteins, lipids, transmitters, Na/K-ATPase etc.
Secondary lysosomes, multivesicular bodies	Retrograde fast component (rFC)	200 mm/d	Similar to aFC

should be associated with different structural alterations.

Experimental studies on axonal transport in toxic and metabolic neuropathies in animal models have so far revealed a variety of changes. In our laboratory, we have examined various experimental conditions in an attempt to recognise patterns of transport abnormalities as well as their relation to neuropathology.¹⁰ We now suggest that three distinct categories of axonal transport abnormalities can be distinguished and related to separate types of nerve pathology.

Impairment of the axonal transport of the anterograde slow component a (SCa)

The anterograde slow components consist of three parts of the cytoskeleton: neurofilaments, neurotubules, and the microfilament network.⁸ Chemically the neurofilaments (SCa), are made up of the so-called neurofilament triplet (200, 145, and 68 kdalton).⁷ The transport velocity of this component in the normal organism is about 1 mm/d. Changes in the transport of neurotubules and microfilaments have not been described and these components will not be mentioned further.

Griffin and his colleagues were the first to suggest that changes in the transport of neurofilaments were related to structural axonal alterations. These authors studied the β , β' -iminodipropionitrile (IDPN) neuropathy and found that swellings of the proximal parts of the axon were due to a retention of neurofilaments.¹¹ Later they showed that distal to the swellings, the neurofilament transport was decreased and atrophy of the axon took place.¹² Similar findings have been made after intoxication with aluminium and 3,4-dimethyl-2,5-hexandione (DMHD).^{13 14} In accordance with these findings quantitative electronmicroscopic studies show a close correlation between the number of neurofilaments (and neurotubules) and axon calibre in normal axons.¹⁵

In experimental diabetes a reduction in the transport velocity of slow component a (SCa) and axonal

dwindling were found.¹⁶⁻¹⁹ Furthermore, we observed that in this condition the amount of material transported was reduced.²⁰ Recently, gelelectrophoretic studies showed a corresponding decreased transport velocity of two neurofilament subunits in diabetic mice.²¹ A decreased transport velocity of SCa and a decrease of fibre calibre are also seen in rats after galactose feeding as well as after administration of BPAU.²²⁻²⁵ Assuming that the transport of SCa is a transport of neurofilaments, this means that axonal atrophy is caused by a decrease of this transport. This conclusion has further been corroborated by the observation that temporary decreases of neurofilament transport induced by acrylamide are followed by temporary reductions of axonal calibre.⁴

In summary, here we have two pathological reactions related to changes in neurofilament transport. At the site of damming of transport a swelling of the axon arises while a decrease of transport leads to axonal atrophy.

Impairment of the axonal transport of the anterograde fast component (aFC)

The anterograde fast component (aFC) consists of membranous material and is presumably transported in the form of vesicles and smooth endoplasmic reticulum.²⁶ The transport velocity is about 400 mm/d and among the many substances contained in the component are transmitters and their enzymes, Na/K-ATPase and many other uncharacterised proteins.⁵ It is assumed that the vesicles are incorporated into the axolemma and the nerve endings and, thus, are necessary for the renewal of the cell membrane of the axon. Therefore, impairment of this component most likely will have rapid and disastrous consequences to the integrity of the axon all along its course.

Ochs suggested several years ago that the transport velocity of this system would be unaffected in all conditions,⁵ as he considered the ATP-dependent mechanism to be an all or none response. We have demonstrated that this holds true as regards experimental

diabetes,²⁷ as well as intoxication with p-bromophenyl-acetylurea (BPAU),²⁴ acrylamide,²⁸ doxorubicin²⁹ and 2,5-hexanedione (2,5-HD).³⁰ In all these conditions transport velocity of aFC remains unchanged.

Glucose is the main fuel for peripheral nerve energy metabolism³¹ and the aFC, therefore, most likely is dependent on the presence of glucose. We have shown that insulin induced hypoglycaemia can lead to acute axonal degeneration with breakdown of axon structure and to characteristic alterations in the cell bodies.³² Recently, we studied the transport during insulin induced hypoglycaemia.³³ Acute lowering of blood glucose leads to reduction in the amount of material in transport whereas velocity is unchanged.

Doxorubicin, a cytostatic agent, interferes with protein metabolism in sensory nerve cell bodies. The neuropathy produced by doxorubicin is characterised by an acute axonal degeneration like the one described in hypoglycaemia (to be published). Again the abnormality in axonal transport is a reduction in the amount of material carried by aFC.²⁹

Though quantitative studies are wanting, colchicine and vincristine are known to impair the fast axonal transport by disruption of the microtubules leaving the slow axonal transport unaffected.³⁴ In human beings as well as in animals axonal degeneration equally distributed along the nerve is associated with the condition.^{35 36}

If a nerve fibre is transected, the axon distal to the interruption undergoes Wallerian degeneration with acute fibre breakdown. The loss of axonal continuity deprives the distal part of the axon of all anterograde components of axonal transport, including SCa. Following the depletion of aFC transport in the isolated distal segment, the amount of material available for retrograde transport is also decreased. Neither reduction in SCa nor in retrograde transport, however, are associated with acute axonal degeneration. Thus, decrease in protein synthesis, decrease in energy level, and mechanical interruption of axon structure are all capable of producing failure in delivery of material by

the fast anterograde transport system and are all associated with degeneration of the acute type.

We, therefore, suggest that the pathological reaction to a decrease in aFC is the acute axonal fibre breakdown of the Wallerian type.

Impairment of the axonal transport of the retrograde fast component (rFC)

The retrograde fast component (rFC) consists of proteins with a molecular composition very similar to the one of aFC.³⁷ The retrogradely transported material is located in multivesicular bodies, the secondary lysosomes.²⁶ Transport velocity is about 200 mm/d and the transport mechanism seems similar to the one of aFC.⁵

It is generally assumed that the retrograde transport can be estimated from the accumulation of material distal to a nerve crush within the first 24 h following fibre interruption. The neuropathies induced by zinc pyridinethione (ZPT),³⁸ BPAU,³⁹ acrylamide,⁴⁰ and 2,5-HD⁴¹ are all characterised as distal axonopathies and the development of symptoms or histological signs takes several days or weeks. In all these toxic conditions the most prominent axonal transport abnormality is by far a reduction of distal accumulation of retrogradely transported material.^{24 40 42-47}

The structural changes of distal axonopathies have not been characterised quantitatively. Preterminal swellings have been observed but it is unknown whether they are primary changes or whether they occur secondarily to loss of the nerve endings. In fact, Mendell and Sahenk cautiously interpreted their findings of a decrease in rFC after administration of ZPT, acrylamide, and 2,5-HD as a secondary phenomenon caused by structural alterations in the nerve endings.⁴⁴

In the BPAU neuropathy, however, reduced distal accumulation is present as early as two days after a single dose is given whereas signs of neuropathy appear several days later.²⁴ Furthermore, the amount of reduction of rFC in disabled animals is closely related to neurophysiological estimates of the degree

Table 2 *Type and causations of peripheral neuropathy in animal models and the associated abnormalities of the axonal transport system*

<i>Axonal transport alteration</i>	<i>Structural consequence</i>	<i>Condition</i>
SCa, impairment	Axonal atrophy/swelling	β , β' -iminodipropionitrile (IDPN), aluminum, 3,4-dimethyl-2,5-hexanedione (DMHD) experimental diabetes, p-bromophenylacetylurea (BPAU), doxorubicin
aFC, decrease of amount	Acute fibre breakdown	Hypoglycaemia, doxorubicin, colchicine, vincristine, Wallerian degeneration
rFC, decrease of amount	Distal axonopathy	Acrylamide, 2,5-hexanedione (2,5-HD), BPAU, zinc pyridinethione (ZPT), (experimental diabetes)
Intact transport	Myelinopathy or electrophysiological disturbance	Diphtheria toxin, tetrodotoxin etc.

of paresis.⁴⁸ In acrylamide neuropathy, the defect in rFC occurs before signs of neuropathy appear and is closely related to the dosage⁴⁴ and the retrograde defect in accumulation cannot be produced by related substances unable to produce neuropathy.⁴⁴ In 2,5-HD intoxication motor functions are more affected than the sensory system and so is the rFC in motor as compared with sensory nerves.⁴⁷ Again the defect is related to dosage and present before neuropathy is apparent.

There is more than one possible explanation of the impairment of retrograde axonal transport. It could be due to a decreased retrograde transport capacity or to a decreased amount of material available for transport in the nerve endings. The latter possibility could occur either as the result of breakdown of aFC in the most distal part of the axon or, more likely, as the result of a defect in the turn-around process.

Whatever the exact mechanism of the impairment of rFC is, it is obvious that this transport abnormality is closely related to the neuropathic condition of the distal axonopathies including withering away of terminals and preterminal swellings with accumulation of vesicular elements and filaments.

Neuropathies without axonal transport abnormalities

As an important corollary of our hypothesis that structural axon changes are mediated by changes in axonal transport, we postulate that if the various components of the axon transport system are unaffected in a neuropathic condition, no structural changes in the axon will be found. Consequently, the neuropathy must either be a myelinopathy or an electrophysiological disturbance.

Conclusion

We suggest that incorporation of the abnormalities of axonal transport in the classification of the different types of nerve pathology is justified by the predictable patterns of transport changes occurring in the various neuropathies. As mentioned above (table 2) three different types of axonal transport abnormalities are now recognised experimentally, namely impairment of aFC and rFC and damming or decrease of SCa.

If this conclusion is valid then in the human condition, distal atrophy due to damming of neurofilament transport might underlie some of the inherited neuropathies; axonal dwindling caused by a decreased neurofilament transport might be seen in early diabetes mellitus; acute axonal degeneration with impairment of aFC might occur in porphyric neuropathy and in hypoglycaemia; while distal axonopathy with a decrease in amount of retrograde axonal transport could represent the type of neuropathy most commonly met after exposure to neurotoxic substances.

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