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Acrylamide-Induced Alterations in Axonal Transport

Biochemical and Autoradiographic Studies

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Contents

Abstract

Alterations in the axonal transport of proteins, glycoproteins, and gangliosides in sensory neurons of the sciatic nerve were examined in adult male rats exposed to acrylamide (40 mg ip/kg body wt/d for nine consecutive days). Twenty-four hours after the last dose, the L5 dorsal root ganglion (DRG) was injected with either [^{35}S]methionine to label proteins or [^{3}H]glucosamine to label glycoproteins and gangliosides. The downflow patterns of radioactivity for [^{35}S]methionine-labeled proteins and [^{3}H]glucosamine-labeled gangliosides were unaltered by acrylamide treatment. In contrast, the outflow pattern of labeled glycoproteins displayed a severely attenuated crest with no alteration in velocity, suggesting a preferential transfer with the unlabeled stationary components in the axolemma. Retrograde accumulation of transported glycoproteins and gangliosides was unaltered for at least 6 h; however, by 24 h, there was a 75% decrease in the amount of accumulated material. The accumulation of [^{35}S]methionine-labeled proteins was not altered. Autoradiographic analysis revealed an acrylamide-induced paucity of transported radiolabeled glycoproteins selectively in myelinated axons with no effect on "nonmyelinated" axons. The pattern of transported proteins was similar in

both control and acrylamide-exposed animals. These results suggest a preferential inhibition of glycosylation or axonal transport of glycoproteins in neurons bearing myelinated axons. More importantly, it suggests that interpretations of axonal transport data must be made with the consideration of alterations in selective nerve fibers and not with the tacit assumption that all fibers in the nerve population are equally affected.

Index Entries: Acrylamide; axonal transport; peripheral neuropathy; glycoproteins; neurotoxicology.

Introduction

Proteins and other cellular macromolecules are synthesized in the neuronal cell body and transported in a continuous fashion to their final destinations in the neuronal processes. This process of axonal transport is necessary for maintenance of the axonal membrane and nerve endings, and alterations in the supply of essential materials are likely to have a detrimental effect on the structure and function of the axon. For example, axonal transport dysfunction has been implicated in the development of many chemically induced peripheral neuropathies. The role of axonal transport in chemical-induced axonopathies has been reviewed extensively elsewhere (Sabri and Spencer, 1980; Brimijoin, 1984; Miller and Spencer, 1985; Sayre et al., 1985; Kristensson and Gustafsson, 1984; Ochs, 1987; Goodrum and Morell, 1992). Since material is carried in both anterograde (cell body to nerve endings) and retrograde (nerve endings to cell body) directions, alterations of transport in either direction could result in an abnormal deposition or accumulation of material along the axon. An alternative possibility is that, under conditions of neuropathy, the metabolic requirements of the axolemma are altered, and this change could be represented by an altered distribution of axonally transported materials.

This article will describe a series of experiments conducted in our laboratory to examine alterations in rapid axonal transport following exposure to acrylamide, a chemical that produces a primary axonopathy. I have obtained data, both biochemical and autoradiographical, suggesting that acrylamide exposure preferentially alters some aspects of glycoprotein transport. I have also observed some of the variables that place limitations on the interpretation of biochemical transport data, i.e., core body temperature, differential response of the nerve to ligation, and the subpopulations of axons comprising the sciatic nerve.

Acrylamide Neuropathy

The peripheral nerves of acrylamide-exposed rats exhibit a multifocal distal axonopathy characterized by axonal swellings at nodes of Ranvier. The axonal swellings are formed by an accumulation of intermediate filaments and other organelles, and are more prominent in the distal regions of the axon (Cavanagh, 1964; Prineas, 1969; Schaumburg et al., 1974; Griffin et al., 1983; Chretien et al., 1981; Le Quesne, 1985). The distal regions of the nerve appear to be more severely affected, and it has been proposed that this is because of less material reaching the distal regions of the nerve as a result of a disruption in axonal transport. Disorders in axonal transport have been implicated in the development of acrylamide-induced peripheral neuropathies (Sabri and Spencer, 1980; Griffin et al., 1983; Jakobsen et al., 1983; Brimijoin, 1984; Miller and Spencer, 1985; Sayre et al., 1985; Spencer et al., 1985; Sabri, 1986; Ochs, 1987; Moretto and Sabri, 1988; Harry et al., 1989).

Rapid Axonal Transport

Much of the literature on the effects of acrylamide on axonal transport appears confusing because of a wide range of dosing models employed producing varying levels of morphological changes in the nerve. Alterations have been reported in rapid bidirectional transport (Sahenk and Mendell,

1981; Jakobsen and Sidenius, 1983; Miller et al., 1983; Miller and Spencer, 1984) with indications that the profile composition of fast-transported proteins is altered and resembles that seen following axotomy (Bisby and Redshaw, 1987). Conflicting reports exist concerning alteration in the velocity of fast transported material. A decrease in velocity has been reported by some (Bradley and Williams, 1973; Sumner et al., 1976; Weir et al., 1978), whereas others report no change from control velocity (Sidenius and Jakobsen, 1983; Harry et al., 1989). Early reports of acrylamide-induced alterations in the rate of rapid anterograde transport could have been confounded by anesthesia-induced hypothermia (Sidenius and Jakobsen, 1983). Investigators normally control for the effects of temperature on axonal transport by maintaining the animal on a constant heat source; however, rarely are core body temperatures monitored. When animals are lightly anesthetized with ether prior to an im injection of ketamine and xylazine, the core body temperature is lowered to 32°C, even in the presence of an external heating source. Under these conditions, the untreated nerve displayed a slight alteration in the downflow profile of radiolabeled proteins (Fig. 1). This was seen as a slight attenuation of the crest, possibly indicative of a minor increase in processing time in the cell bodies prior to commitment of newly synthesized material to transport. In contrast, an identical low core body temperature in acrylamide-exposed rats resulted in a pronounced alteration in the downflow profile of rapidly transported radiolabeled proteins in a pattern suggesting a decrease in velocity (Fig. 1). Although both acrylamide and control animals maintained identical core body temperatures of 32°C, a differential effect was seen on transport rate in the acrylamide-exposed nerve. When core body temperatures were not lowered with anesthesia (ketamine and xylazine used in the absence of ether), no acrylamide-induced alteration in rapid transport rate was seen (Harry et al., 1989).

An additional possibility for the conflicting reports on axonal transport following acrylamide exposure is the choice of radiolabeled precursor.

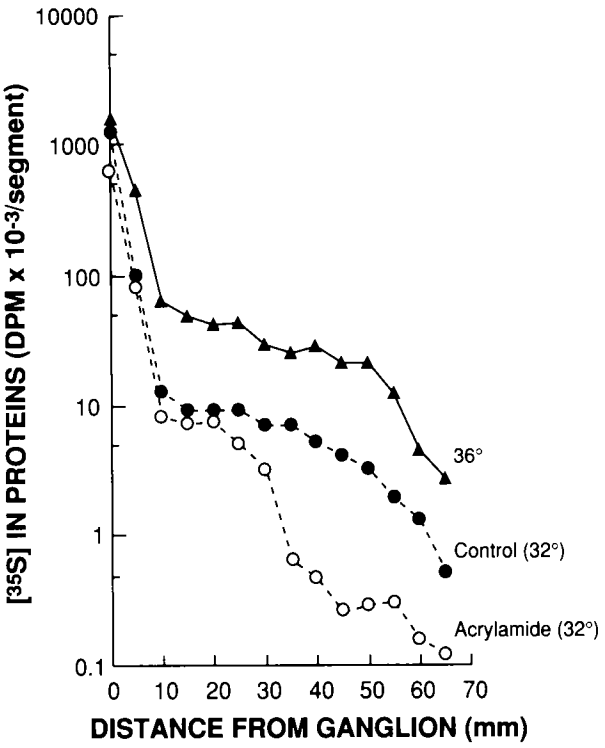

Fig. 1. Temperature-related alterations in transport profiles of newly synthesized proteins. Representative distribution of labeled proteins in the DRG and sciatic nerve 5 h after injection of 100 μCi [^{35}S]methionine (1,131 Ci/mmol) into the L5 ganglion. Representative downflow profiles illustrate the movement of the initial crest of transported labeled material down the nerve. Radioactivity present in consecutive 5-mm segments (abscissa) is given on the ordinate in a logarithmic scale. Both acrylamide-exposed and control animals preanesthesized with ether prior to ketamine/xylazine anesthesia, maintained a rectal core body temperature of 32°C for approx 2 h postsurgery. All animals receiving only ketamine/xylazine maintained a normal rectal core body temperature. All animals were maintained on a heated surface postoperatively. Because of the large amount of radiolabeling of nonneuronal cells within the DRG, the DPMs at zero distance from the ganglion were not normalized for plotting purposes. Normalization did not alter the shapes of the transport profiles.

Using an acrylamide dosing regimen (40 mg ip/kg body wt/d for nine consecutive days), which produced clinical signs of hindlimb splay and weakness in the absence of gross morphological alterations, the transport of newly synthesized proteins labeled with [^{35}S]methionine was unal-

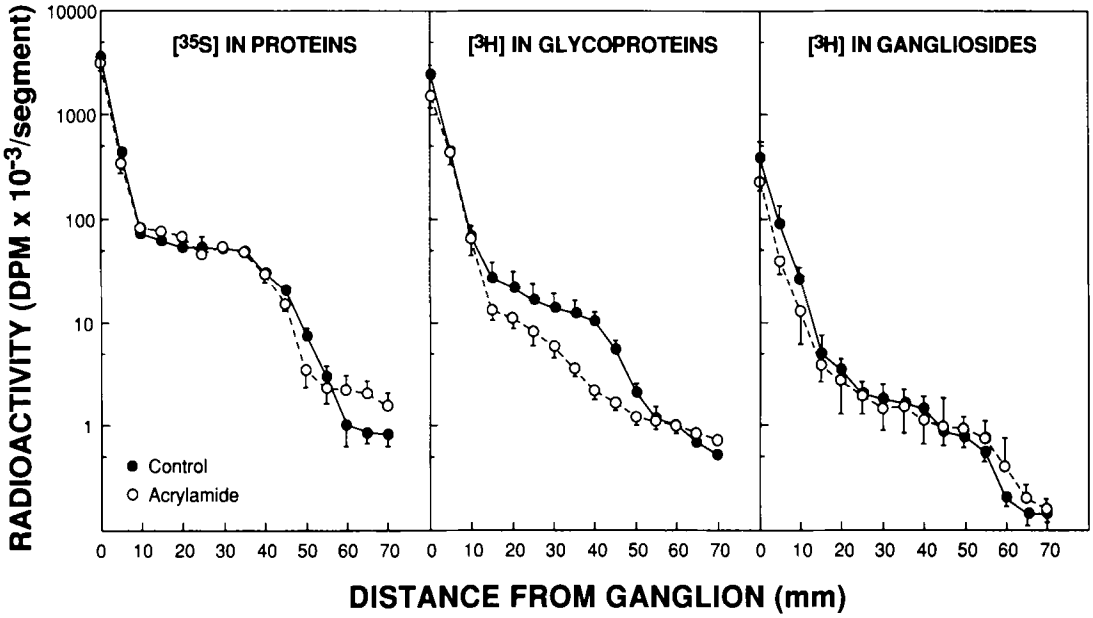

Fig. 2. Distribution of labeled material in the DRG and sciatic nerve of control and acrylamide-exposed rats at 4 h following injection of either 100 μCi [^{35}S]methionine (1,131 Ci/mmol, New England Nuclear, Boston, MA) or 200 μCi [^{3}H]glucosamine hydrochloride (20 Ci/mmol, New England Nuclear) into the L5 DRG. Downflow profiles, each representing the mean ± SEM of a minimum of six rats are shown to illustrate the movement of the initial crest of transported labeled material down the nerve. Radioactivity present in consecutive 5-mm segments (abscissa) is given on the ordinate in a logarithmic scale. The approximate position of the initial crest of transported radioactivity was determined by visual inspection as the most distal 5-mm segment of the plateau region. Normalization of the DPMs in the DRG did not alter the shapes of the transport profiles and was not used for plotting purposes.

tered (Harry et al., 1989). The transport downflow profile, for both control and acrylamide-exposed animals, displayed a well-defined crest (45 mm distal to the dorsal root ganglion [DRG]) of rapidly transported material (Fig. 2). However, acrylamide exposure altered the kinetics of fast anterograde transport of [^{3}H]glucosamine-labeled glycoproteins as displayed by a severe attenuation of the crest of transported material (Fig. 2). When a defined crest could be identified, the maximal transport rate was calculated by the method described by Ochs (1972) based on the displacement of the crest (ganglion to crest distance) as a function of time after injection. In the absence of a defined crest, the maximal rate for anterograde transport was based on the displacement of the fastest moving edge of transported material. An additional determination of transport rate for glycoproteins and gangliosides was determined with data from a double-ligature method by a modification (Harry et al., 1987) of the procedure of Rasool and Bradley (1978). Regardless of radioactive precursor, class of molecule, or calculation method, transport rates in acrylamide-exposed animals were similar to that in normal animals. The observation of a normal transport rate coupled with an attenuated crest of rapidly transported material suggests a preferential transfer of transported glycoproteins from the transport vector to stationary axonal structures.

To examine further the selective effect of acrylamide on glycoprotein transport, I injected an isotope mixture of 200 μCi D-[6-^{3}H]glucosamine hydrochloride and 25 μCi [^{35}S]methionine into the L5 DRG. A ligature was immediately placed on the sciatic nerve approximately 30 mm distal to the DRG. Transported material was

allowed to accumulate proximal to the ligature for 4 h. This accumulated material represented the initial edge of rapidly transported labeled material. The nerve was excised and dissected into consecutive 5-mm segments with a 2-mm accumulation segment immediately proximal to the ligature and radioactivity within each segment determined. The accumulation of [^{35}S]methionine-labeled proteins at the ligature was similar for both control and acrylamide-exposed animals. However, acrylamide exposure decreased, by one-half, the amount of [^{3}H]glucosamine-labeled glycoproteins accumulated (data not shown).

The amount of labeled material remaining in the axolemma behind the crest of rapidly moving material was examined by ligating the sciatic nerve (3 mm distal to the DRG) 2.5 h following precursor injection and examining the downflow profile of radioactivity 3 h later. Acrylamide exposure increased the amount of label remaining in the axon as indicated by a decrease in the pulse of radioactivity moving down the axon (Harry et al., 1989). Thus, these two experiments supported the hypothesis of increased deposition of transported glycoproteins.

Retrograde Transport

Since the distal portion of the peripheral nerve displays a greater sensitivity to acrylamide exposure, it has been of interest to examine the process of axonal transport in these distal areas. A number of events take place in the distal axon, including anterograde delivery, turnaround, and retrograde return of labeled materials. Several investigators have reported alterations in retrograde transport following acrylamide exposure. A single dose of acrylamide has been shown to impair retrograde transport in sensory and motor axons (Miller and Spencer, 1984; Sabri, 1986) as well as induce a dose-dependent deficit in the arrival of retrogradely transported [^{125}I]nerve growth factor (Miller et al., 1983), horseradish peroxidase (Kemplay and Cavanagh, 1983), and tetanus toxin (Miller and Spencer, 1984; Moretto and Sabri, 1988) in the DRG. These types of studies rely on the endocytosis, by the nerve terminals, of an exogenously applied marker. Therefore, for proper interpretation, the effects of acrylamide on retrograde transport are distinguished from those of marker uptake. A separate approach to examining retrograde transport involves the use of ligatures placed upon the nerve (Bisby, 1977; Bisby and Bulgar, 1977). In this procedure, the cell body is injected with a radiolabeled precursor, and the newly synthesized material is allowed to be transported along the length of the nerve. Following a period of time, two ligatures are placed on the sciatic nerve of a rat approx 45 and 60 mm from the DRG, respectively. Over time, the amount of material that accumulates proximal to the proximal ligature represents anterograde transport, whereas that which accumulates distal to the distal ligature represents retrograde transport. Such ligation data are customarily used to calculate relative accumulation values to illustrate the time-course of anterograde and retrograde transport (Bisby, 1977). A number of investigators have utilized this procedure to examine toxicant-induced alterations in retrograde transport. Acrylamide has been reported to decrease the rate at which material is retrogradely transported (Sahenk and Mendell, 1981). However, with similar procedures, Jakobsen and Sidenius (1983) concluded from their data that neither transport velocity nor "turnaround" time was altered following acrylamide exposure. Instead, they interpreted their data as representing a deficit in the amount of material carried by retrograde transport.

I have utilized this double-ligature method to examine different aspects of transport following exposure to acrylamide. Briefly, the DRG was injected with either [^{3}H]glucosamine or [^{35}S]methionine. At intervals of either 6 or 18 h after injection, rats were again anesthetized, the right sciatic nerve exposed in the midthigh region, and two silk ligatures applied 14 mm apart, approx 35 and 50 mm distal to the injected ganglion. Following a specified time of accumulation, the nerve was excised and dissected

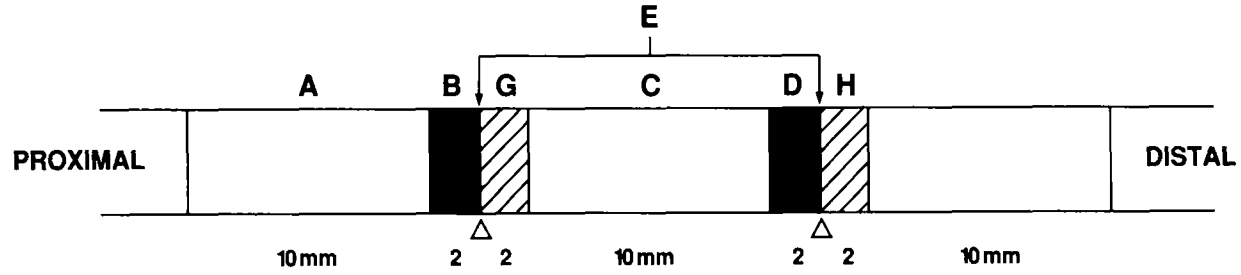

Fig. 3. A, average baseline dpm/mm for 10-mm segment; B, total anterograde dpm accumulated at proximal ligature (2 mm); C, average dpm/mm in central nonaccumulation (9–12 mm) segment; D, total anterograde dpm accumulated at distal ligature (2mm); E, total dpm between ligatures; F, length of accumulation segment (2 mm); H, total retrograde dpm accumulated at distal ligature (2 mm); G, total retrograde dpm between ligatures accumulated at proximal ligature (2 mm); T, hours of ligature duration. Percent of mobile anterograde = I = [D – (F • C)]/E; % mobile retrograde = [T – (F • C)]/E. Anterograde rate = [B – (F • C)]/T • A • I. Relative Accumulation (RA) = (radioactivity/mm in accumulation segment)/estimated baseline. Estimated baseline = summed radioactivity in bracketing segments (10-mm segment either proximal or distal to accumulation segment and total segment between ligatures)/summed length of bracketing segments. ■ Anterograde accumulation; ▨ retrograde accumulation; Δ ligatures.

Table 1
Relative Accumulation of Transported Radiolabeled Glycoproteins, Gangliosides, and Proteins[a]

Pre/Post ligature		Glycoproteins		Gangliosides		Proteins	
		Control	Acrylamide	Control	Acrylamide	Control	Acrylamide
6/4	AA	13.1 ± 0.9	10.0 ± 1.9	11.7 ± 1.2	7.9 ± 2.1		
	RA	1.8 ± 0.7	1.5 ± 0.9	3.0 ± 0.6	2.4 ± 1.1		
18/6	AA	4.4 ± 0.5	4.4 ± 0.9	4.0 ± 1.0	4.4 ± 0.9	4.3 ± 0.1	3.0 ± 0.9
	RA	3.0 ± 0.1	3.2 ± 0.6	2.8 ± 0.7	2.7 ± 0.5	1.1 ± 0.1	2.1 ± 0.1
18/24	AA	7.3 ± 0.4	6.5 ± 1.0	9.1 ± 0.8	5.9 ± 1.6	4.7 ± 0.4	5.2 ± 0.5
	RA	11.2 ± 1.3	2.4[b] ± 0.7	5.6 ± 1.5	1.9[b] ± 0.5	4.1 ± 0.6	3.7 ± 0.4

[a]Values are means ± SEM (N = 6 animals/group)
[b]$p < 0.05$ by Student's t-test.

according to the design represented in Fig. 3. Relative accumulation values for both anterograde and retrograde transport were calculated according to the methods developed by Bisby (1977) and detailed in Fig. 3.

Analysis of the accumulation of labeled material at ligatures indicated that the early appearance, within 4–6 h, of material transported in both the anterograde and retrograde direction was similar for control and acrylamide-exposed animals (Table 1). When material was allowed to accumulate for 24 h, no alteration was seen for anterograde transport; however, a 75% decrease in the amount of retrogradely accumulated [^{3}H]glucosamine-labeled glycoproteins or gangliosides was seen in acrylamide-exposed animals (Table 1). Similar effects were seen with [^{3}H]fucose-labeled glycoproteins. No alterations were seen in the accumulation of [^{35}S]methionine-labeled proteins. Effects seen at the longer accumulation time-point may not represent the retrograde transport process itself, but instead may be owing to the mechanical perturbation of the ligature on a previously compromised nerve.

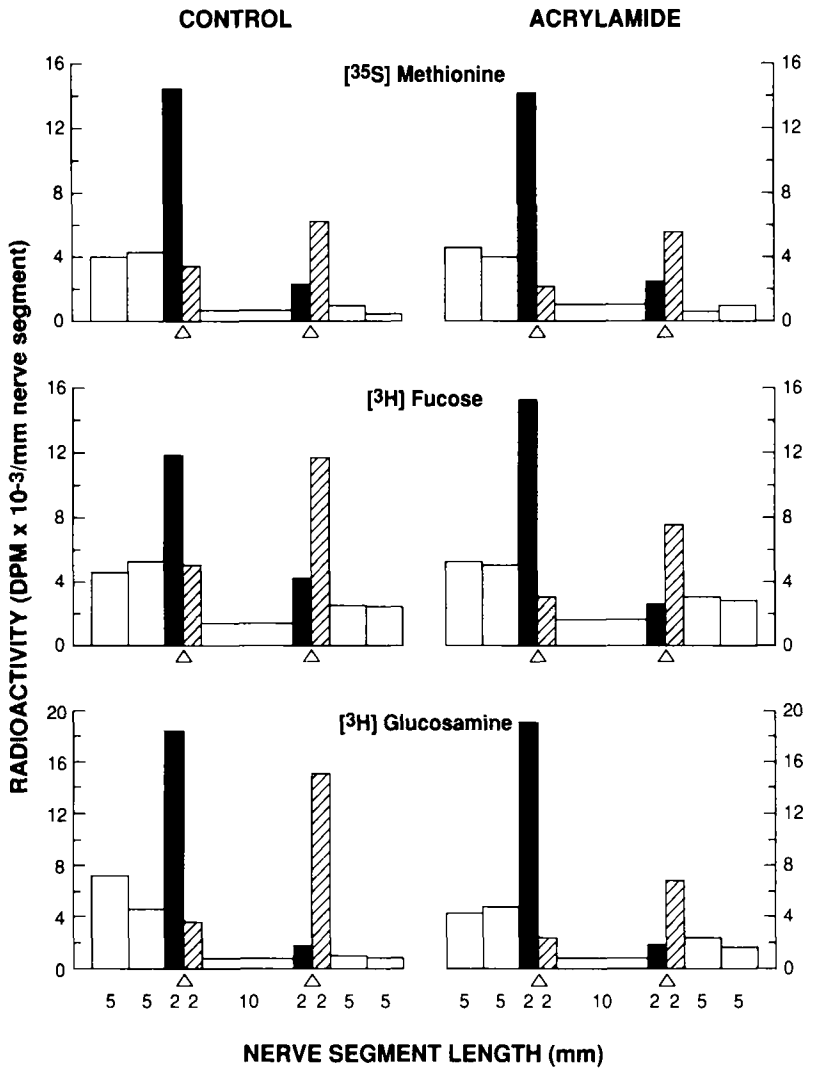

Fig. 4. Representative profiles of the distribution of [^{35}S]methionine labeled proteins [^{3}H]fucose- or [^{3}H]glucosamine-labeled glycoproteins in ligated sciatic nerve. Eighteen hours following injection of radiolabeled precursor into the L5 DRG, two ligatures (▲) were placed on the sciatic nerve approx 35 and 50 mm distal to the DRG. Transported radioactivy was allowed to accumulate for 24 h.

The ligature may either accelerate the ongoing degenerative process or initiate a local nerve degeneration producing a structural or biochemical change, resulting in less material available for retrograde transport. In support of this interpretation, it has been demonstrated that when a ligature was placed on the sciatic nerve, acrylamide exposure resulted in a "dying-back axonopathy" from the site of ligation (Cavanagh and Gysbers, 1980) and that the sciatic nerve of an acrylamide-exposed animal was more susceptible to injury from ligation (Sharer and Lowndes, 1985). The most severe degeneration was seen closest to the ligature, and the level of degeneration increased with the distance from the DRG (Sharer and Lowndes, 1985).

The pattern of movement between the ligatures (Fig. 4) indicated no treatment effect on the percent of the total radiolabeled material (proteins, glycoproteins, or gangliosides) that was

moving in either the anterograde or retrograde direction—implying that the mechanisms of transport were intact. Relative to the total amount of labeled material between the ligatures, the percentage of gangliosides and glycoproteins in the stationary component (as represented by radiolabeled material remaining in the one long midsection between the ligatures) was doubled in acrylamide-treated nerves (control—33%; acrylamide—60%) over a 24-h time period. This increase in the stationary component may be representative of either the deposition of radioactivity or a redistribution of radiolabel in the area between the ligatures. These data suggest that the observed effect on retrograde accumulation may be the result of an alteration in the amount of [^{3}H]glucosamine-labeled material available for retrograde transport as a result of an increased deposition/exchange of labeled material in the distal axolemma. Again, these data are consistent with our anterograde transport studies suggesting increased deposition of glycoproteins.

Autoradiographic Studies of Transport

All the above data are consistent with the hypothesis that the decreased amount of transported glycoproteins reaching the distal axonal regions in acrylamide-exposed animals is related to an increased unloading during transit of rapidly transported glycoproteins from the transport vector onto stationary axonal structures. In order to evaluate directly the increased deposition of newly synthesized glycoproteins, we used autoradiographic techniques to examine alterations in the distribution pattern of rapidly transported labeled material (Harry et al., 1992). The distribution pattern of silver grains was examined within the sciatic nerve of control and acrylamide-exposed rats 5 h following precursor injection into the DRG. In control animals, silver grains representing, predominantly, radiolabeled proteins or glycoproteins were often aligned in trails over the myelinated axons and frequently aggregated at nodes of Ranvier (Fig. 5). The silver grains outside the myelinated axons also aligned in trails that paralleled the known course of unmyelinated axons. Only a fraction of the axons contained silver grains, which is representative of the limited contribution of axons in the sciatic nerve originating from cell bodies of the L5 DRG. These patterns are consistent with the axonal transport of the labeled material rather than uptake of blood-borne label by interstitial cells.

In acrylamide-exposed animals, the distribution pattern of silver grains representing rapidly transported proteins labeled with [^{3}H]methionine was similar to that in controls. For [^{3}H]glucosamine-labeled nerves, a very distinct alteration was seen in acrylamide-exposed animals. Silver grains were seen over the nonmyelinated regions, presumably in unmyelinated axons; however, there was a paucity of grains over myelinated axons (Fig. 6). The altered ratio of label in myelinated axons relative to that in areas of unmyelinated axons was apparent by visual inspection.

Quantitative analysis of the distribution pattern of silver grains verified the visual inspection. For each nerve segment, 24 defined regions were examined, and an average of each parameter was generated. Approximately 25 myelinated axons were contained in each region. As presented in Table 2, no acrylamide-induced alterations were seen in the distribution pattern of [^{3}H]methionine-labeled proteins. When [^{3}H]glucosamine was used as a precursor, a significant decrease was seen in the percentage of myelinated axons containing silver grains as well as the number of grains in myelinated axons. This altered distribution of labeled glycoproteins within the sensory axons of the sciatic nerve was found in all acrylamide-exposed animals examined and was evident in all segments of the sciatic nerve at various distances distal to the DRG. The number of grains in "nonmyelinated" regions was not altered, suggesting that a normal amount of newly synthesized material is transported in the unmyelinated axons of acrylamide-exposed animals.

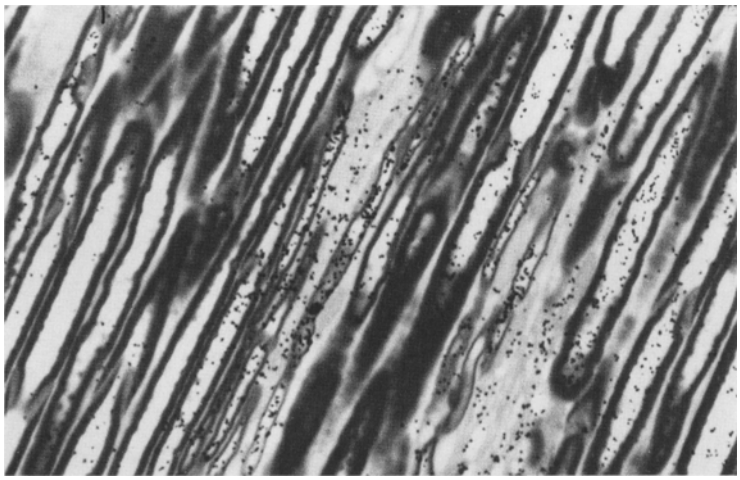

Fig. 5. Longitudinal section of right sciatic nerve from control rat that received an injection of 400 μCi D-[6-^{3}H]glucosamine hydrochloride in right L5 DRG 5 h prior to removal of nerve for autoradiography. White, unstained axons are outlined by their black myelin sheaths. Black silver grains, which represent rapidly transported labeled glycoproteins, are over some, but not all, myelinated axons. Silver grains are outside the myelinated fibers in areas normally occupied by unmyelinated axons. Consistent with the small contribution of myelinated and unmyelinated axons in the sciatic nerve from the neurons of the L5 DRG, silver grains were found in only a portion of the total number of axons. All control nerves displayed similar patterns regardless of precursor. Paraphenylenediamine stain: 900×.

A New Hypothesis

I have reevaluated this biochemical data of axonal transport (downflow profiles, ligature data) in light of the autoradiographic profiles of silver grain distribution. All of the previous biochemical literature concerning alterations in axonal transport in perturbed systems, including our own, assumed that all fibers in the nerve population were being equally affected. It had been shown, for example, that the rate of rapid anterograde transport is similar for both myelinated (Ochs, 1972) and unmyelinated axons (Ochs and Jersild, 1974) of the sciatic nerve. Whether the transport profiles are similar between these two axonal populations is not known. A review of our data concerning acrylamide-induced alterations in the transport kinetics of labeled glycoproteins (Harry et al., 1989) reveals an abnormality characterized by a marked attenuation of the initial crest of transported material. Such profiles have been interpreted as indicating an increased unloading of rapidly transported material from the transport vector onto stationary axonal structures (Blaker et al., 1980; Harry et al., 1987), since there should always be some transfer of lipids and proteins to account for the metabolic turnover observed for almost all biological components (Toews et al., 1988). The autoradiographic localization of silver grains demonstrated that the radioactivity represented in the biochemical downflow profiles of acrylamide-exposed animals is contained almost exclusively in the "nonmyelinated" regions of the nerve. Thus, the finding of an attenuated crest in the downflow profile may reflect an unmasking of the normal physiological situation in unmyelinated fibers. It is possible that the metabolic activity of ion transport and conduction of action potentials along the entire length of the axolemma may require a greater turnover of glycoproteins in the unmyelinated axon. This could result in a greater demand for a transfer of glycoproteins from the transport vector to the glycoprotein-enriched axolemma, resulting in an attenuated downflow profile for these fibers.

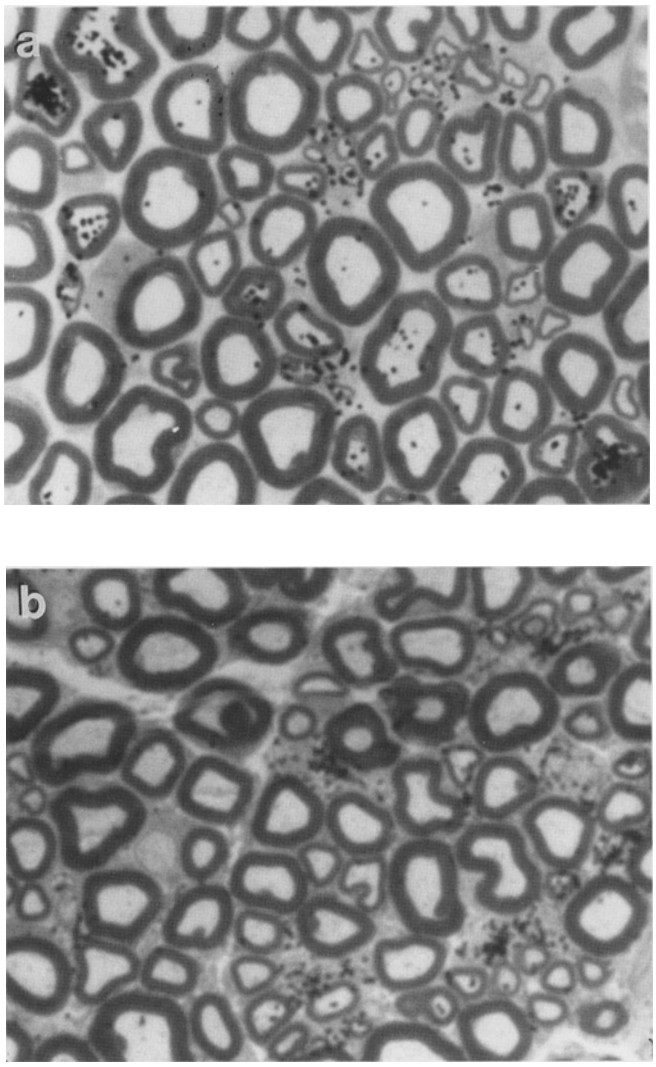

Fig. 6. Cross-sections of sciatic nerve from control (A) and acrylamide-exposed (B) rats that received a 1-μL injection of 400 μCi D-[6-^{3}H]glucosamine hydrochloride in the right L5 DRG 5 h prior to removal of nerve for autoradiography. The black silver grains represent labeled material rapidly transported in myelinated and unmyelinated sensory axons. In controls, a large number of silver grains representing labeled glycoproteins are seen over myelinated fibers and over areas occupied by unmyelinated fibers. This distribution pattern is similar to that seen in longitudinal sections. Sciatic nerves from acrylamide-exposed rats show a large number of silver grains over areas of unmyelinated axons, but a paucity of silver grains over the myelinated axons. Paraphenylenediamine stain: 1050×.

The basis for the preferential vulnerability to acrylamide of glycoprotein transport in neurons that support myelinated axons is not known. The paucity of grains in myelinated axons points to effects within the cell body. Morphological alterations occur in neuronal cell bodies as an early response to acrylamide exposure (Cavanagh, 1982; Sterman, 1983) with a preferential involvement of the larger cell bodies (Jones and Cavanagh, 1984). These larger perikarya may be associated with the larger myelinated axons, since the sizes of both the neuronal perikaryon and its axon are related to the size of the peripheral projection field (Donaldson and Nagasaka,

Table 2
[^{3}H] Glucosamine or [^{3}H]Methionine-Labeled Axonally Transported Material in Myelinated Axons of Rat Sciatic Nerve[a]

	# Grains myelinated axons	# Grains nonmyelinated regions	# Grains myelinated/ nonmyelinated	% of Myelinated axons w/grains
[^{3}H] glucosamine				
Acrylamide	9.8[b] ± 1.7	32.8 ± 5.6	0.31[b] ± 0.07	9.8[b] + 1.2
Control	30.0 ± 3.3	31.0 ± 4.8	1.29 ± 0.18	35.5 ± 3.8
[^{3}H]methionine				
Acrylamide	21.8 ± 2.12	23.8 ± 2.0	0.93 ± 0.10	33.0 ± 5.5
Control	24.0 ± 2.7	23.8 ± 4.1	1.05 ± 0.09	37.2 ± 4.9

[a]Values are the mean ± SEM; $N = 6$.
[b]$p \leq 0.01$ by Student's t-test relative to corresponding control.

1918; Lieberman, 1976). Under normal conditions, newly synthesized proteins are compartmentalized in the neuronal perikarya in preparation for axonal transport, with a subset of proteins being glycosylated in their transit through the endoplasmic reticulum and Golgi complex (for reviews, *see* Droz, 1969; Lasek, 1970; Leblond and Bennett, 1977; Stone and Hammerschlag, 1987; Goodrum et al., 1989). Much of the early cell body response to acrylamide involves disruption of the Golgi complex and the endoplasmic reticulum (Cavanagh, 1982; Sterman, 1983). This cellular response could result in an alteration in a processing step at the level of glycosylation or further along at the level of commitment of glycoproteins to rapid transport down myelinated axons, thus accounting for the specificity of the acrylamide effect on glycoprotein transport. Preliminary autoradiographic examination at the light microscopic level indicated no acrylamide-induced alteration in silver grain distribution or amount in DRG cell bodies. It did, however, reveal that a number of various cell types other than neuronal cells within the ganglion are heavily labeled with each precursor. This places into question the practice of normalizing across DRGs to control for injection variability or differences in incorporation of label. In the same way that the radioactivity in the initial nerve segments (approx 10 mm from the DRG) is considered to represent transported material and nontransported material owing to leakage from the injection site, the radioactivity in the DRG is not representative solely of incorporation or radiolabeled precursor into the neuronal cell bodies. For these reasons, I did not normalize the transport data for plotting purposes.

Summary

In the first paper concerning acrylamide-induced alterations in axonal transport (Harry et al., 1989), I proposed a number of hypotheses to explain the observed alteration in the kinetics of glycoprotein transport. One hypothesis proposed that acrylamide exposure may preferentially damage axolemmal glycoproteins, resulting in an increase in unloading or exchange of newly synthesized glycoproteins in the proximal portion of the axon. This was consistent with the proposal of others that acrylamide may act directly on the axon, alter the metabolic requirements of the axolemma (Spencer et al., 1978; Brimijoin and Hammond, 1985), and increase unloading of transported material along the axon (Bradley and Williams, 1973; Chretien et al., 1981; Souyri et al., 1981). Accumulation of material could also occur in

the proximal portion of the sciatic nerve, where early morphological alterations and structural disorganization of the nodal-paranodal regions have been demonstrated (Brismar et al., 1987). In almost all of the previous biochemical literature concerning alterations in axonal transport in perturbed systems, the interpretations of the data have been made with the tacit assumption that all fibers in the nerve population were being equally affected. The autoradiographic data examining distribution of silver grains showed a preferential effect of acrylamide on a subpopulation of axons, with a paucity of [^{3}H]glucosamine-labeled material in myelinated axons. The possibility of such selective alterations must be taken into consideration when interpreting axonal transport data. These data, with regard to anterograde transport, have also shifted the focus of the injury away from axonal transport *per se* to events within the cell body. The lack of delivery of glycoproteins to the distal regions of the myelinated axons may account for the pathological structural alterations that eventually appear following exposure to acrylamide. These findings do not dispute the effects of acrylamide on retrograde transport. They may, in fact, present a framework for future examination. A selective nerve fiber perturbation may also exist with regard to retrograde transport. It may be possible by a reexamination of the literature, in light of differential effects with regard to radiolabeled precursors and subpopulations of fibers within the nerve, to establish a cohesive story for the role that axonal transport plays in the pathogenesis of acrylamide-induced distal axonal degeneration.

Acknowledgments

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