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Experimental Diabetic Neuropathy: Similar Changes of Slow Axonal Transport and Axonal Size in Different Animal Models

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Analysis of slow axonal transport in sciatic and primary visual systems of BB rats with spontaneous diabetes of 2.5–3.5 months duration revealed a delay in transport of the neurofilament (NF) subunits, tubulin, actin, and the 60, 52, and 30 kDa polypeptides in both systems. The polypeptides examined were not affected uniformly. Rather, the transport of the 60, 52, and 30 kDa polypeptides and the rapidly moving component of tubulin, all constituents of the slow component b (SCb) of axonal transport, appeared to be more severely delayed than the transport of polypeptide constituents of the slow component a (SCa), such as NF and the slow-moving tubulin. Transport was not impaired in diabetic BB rats maintained normoglycemic with optimal doses of insulin. A 52 kDa polypeptide constituent of SCb was identified as neuron-specific enolase, and the 30 and 60 kDa polypeptides are likely to be aldolase and pyruvate kinase; all 3 are glycolytic enzymes. Morphometric analysis revealed that the cross-sectional area of sciatic axons was increased proximally at the level of the motor roots and decreased distally at the level of the tibial nerve. The changes in slow transport and caliber observed in central and peripheral axonal systems of diabetic BB rats are virtually identical to those previously described in rats with streptozotocin-induced diabetes, another model of insulin-dependent diabetes. In both models, the alterations of axonal caliber are likely to be secondary to the impairment of axonal transport. The presence of the same type of impairment of axonal transport in association with the same alterations of axonal caliber in 2 different experimental models of insulin-dependent diabetes indicates that these changes are specifically associated with this type of diabetes and are also likely to occur in human diabetes. The marked transport impairment of glycolytic enzymes and/or other constituents of SCb may lead to the distal degeneration of axons that characterizes the advanced stages of diabetic neuropathy.

The axon is entirely dependent on the cell body for the supply of all its constituents, which are continuously conveyed by axonal transport (Grafstein and Forman, 1980; Lasek et al., 1984). Axonal transport, therefore, plays a basic role in maintaining the structural integrity of the axon, and its impairment may be the primary event in several human neuropathies, including those characterized by axonal degeneration.

Axonal degeneration is considered the hallmark of the human diabetic polyneuropathy and the main cause of functional impairment (Brown and Ashbury, 1984; Thomas and Eliasson, 1984). The pathogenetic mechanisms of diabetic neuropathy and the question of whether these mechanisms include an impairment of axonal transport remain to be defined. Three main mechanisms have been proposed: (1) alteration of endoneurial vessels, leading to widespread anoxia or multiple infarcts (see Low, 1987, for review); (2) metabolic abnormalities that include reduction of free myoinositol, Na^+K^+-ATPase activity, and rate of protein synthesis (see Low, 1987, for review); and (3) direct alteration of proteins by nonenzymatic glycosylation (Kennedy and Baynes, 1984; Panush Cohen, 1986). Each of these mechanisms could lead to axonal degeneration by impairing axonal transport or causing a direct injury to the axon. In order to define the role of axonal transport in diabetic neuropathy, the first issue that must be resolved is whether changes in axonal transport sufficiently severe to cause axonal degeneration are consistently associated with diabetes.

Previous experimental studies on axonal transport using animal models of diabetes have provided contradictory results (Bisby, 1980; Jakobsen and Sidenius, 1980; Brimijoin, 1982; Jakobsen et al., 1983). In a recent study we have shown that slow transport is similarly impaired in central and peripheral axons of rats with insulin-dependent diabetes induced by streptozoticin (SZ) (Medori et al., 1985). Individual polypeptides seemed to be affected selectively. Polypeptides transported with slow component b (SCb), which include the rapidly moving component of tubulin and several enzymes of intermediary metabolism, such as aldolase, pyruvate kinase (PK), neuron-specific enolase (NSE), and creatine phosphokinase (CPK), were delayed more than neurofilament (NF) polypeptides and the slow-moving component of tubulin, which are the main constituents of slow component a (SCa) (Lasek et al., 1984). In the same animals, the axonal caliber was increased proximally at the level of the motor roots and decreased distally at the level of the tibial nerve (Medori et al., 1985).

In this study we report that the same changes of slow transport and axonal caliber as those observed in rats with SZ-induced diabetes occur in the diabetic BB rat, a model for spontaneous insulin-dependent diabetes with many homologies to human diabetes (Rossini et al., 1985). This finding establishes that impairment of slow transport with alterations in axonal caliber is consistently associated with insulin-dependent diabetes in animal models and suggests that similar changes occur in human

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diabetes. Parts of this study have been presented previously (Medori et al., 1986a–c).

Materials and Methods

Animals. Age-matched diabetes-prone and diabetes-resistant male Wistar rats were obtained from the NIH-sponsored BB/Wor colony maintained at the University of Massachusetts Medical School. Diabetes-prone rats had hyperglycemia and glycosuria when they were received and were given standard rat food (Purina Chow) and drinking water containing 400 mg/liter of tetracycline ad libitum. Weight, as well as nonfasting urinary glucose and ketone levels, was measured daily. Insulin (Ultralent Zinc-Protamin) diluted 10 U/ml of sterile saline solution was administered subcutaneously to diabetic rats in amounts adjusted to maintain a permanent glycosuria with neither ketonuria nor weight loss. Occasional ketonuria was corrected by increasing the dose of insulin and by administering Ringer's lactate (5–10 ml) hypodermically. Blood glucose levels were determined weekly in rats that had fasted for 4 hr. Two types of control animals were used: (1) diabetic-resistant rats, housed and controlled for blood glucose levels in the same manner as the diabetic rats; (2) diabetic-prone rats, initially maintained as hyperglycemic like the rats of the experimental groups and later treated with 2 daily administrations of insulin in doses adjusted to maintain normal glucose levels and suppress glycosuria and ketonuria. We refer to these 2 types of controls as nondiabetic and diabetic–normoglycemic rats, respectively.

Axonal transport. ^{35}S-Methionine was administered either by intraocular or intraspinal injection (500 μCi in 4.5 μl of sterile saline, 750 μCi in 3 μl of sterile saline, respectively) (Papasozomenos et al., 1982; Medori et al., 1985; Monaco et al., 1985). Animals were killed by intraaortic perfusion of saline, 25 and 42 d following the intraocular and 28 d following the intraspinal injection. All animals were killed at 6–10 months of age. At the time of ^{35}S-methionine administration, diabetic animals had had hyperglycemia for 2.2–3.2 months. The diabetic–normoglycemic animals were kept hyperglycemic for at least 1.5 months; then levels of glucose and ketones in urine and of glucose in blood were maintained normal for at least 1 month before ^{35}S-methionine administration. Primary visual and sciatic systems were promptly dissected out and cut into 3 mm segments, except for the chiasm, which measured 2 mm. Segments were homogenized individually in electrophoresis sample buffer, total radioactivity determined in an aliquot by liquid-scintillation counting, and the remainder processed for one-dimensional PAGE (1D-PAGE) and fluorography (Bizzi et al., 1984; Medori et al., 1985; Monaco et al., 1985). The radioactivity related to the NF subunits (200, 145, and 68 kDa), tubulin, actin, and polypeptides of 20, 30, 52, and 60 kDa was estimated by integration of the corresponding areas in scans of the 1D-PAGE fluorograms obtained with a computer-assisted laser scanner (LKB Ultroscan XL) (Hoffman et al., 1983; Medori et al., 1985; Monaco et al., 1985). The percentage of the total radioactivity related to a polypeptide was expressed as a function of the distance from the eye or from the spinal cord. Transport rates of selected polypeptides were obtained by determining the location of the 50th percentile of radioactivity (Hoffman et al., 1983; Medori et al., 1985). Data were obtained from groups of 3 diabetic and 3 control rats for each experiment. A 2-tailed Student t test was used for statistical analysis of the data.

Labeled polypeptides present in the proximal and distal segments of the optic system of diabetic and control rats were analyzed by 2D-PAGE and nonequilibrium pH gel electrophoresis (NEPHGE) (O'Farrell, 1975; O'Farrell et al., 1977; Autilio-Gambetti et al., 1982). The proteins in some of these gels were transferred to nitrocellulose (Towbin et al., 1979) and incubated with antiserum to rat neuron-specific enolase (NSE) (Polyscience, Warington, PA), and the reaction visualized by the immunoperoxidase method. The nitrocellulose membrane was then exposed to XAR film to obtain autoradiograms.

Morphometric analysis. The sciatic systems from 3 rats with 10–12 week diabetes and 3 nondiabetic controls were analyzed. Animals were fixed by intraaortic perfusion with 5% buffered glutaraldehyde, as previously described (Medori et al., 1985). The entire sciatic system was cut into 5 mm segments that were fixed overnight, postfixed in 2% OsO_4 and embedded in Spurr resin. Sections, 1 μm thick, 10–15 and 120–125 mm from the spinal cord, were chosen for morphometric analysis. The size of axons was determined by a computer-assisted digitizing system, and histograms of percentage distribution of axons as a function of axonal size were obtained (Medori et al., 1985). The Kolmogorov-Smirnov 2-group test was used for statistical analysis of the data.

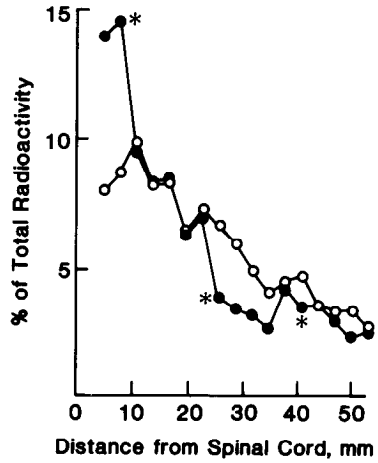

Figure 1. Distribution of the total radioactivity present in the sciatic system, 28 d following intraspinal injection of ^{35}S-methionine. Control animals (○) show a peak at 12 mm that corresponds to SCa, while the bulk of the radioactivity migrating with SCb is in segments located 23–50 mm from the spinal cord. In diabetic rats (●), the bulk of the radioactivity is located within the proximal 9 mm root segment and the radioactivity in SCb is markedly reduced. *$p < 0.05$.

Results

Diabetes

In diabetic rats, blood glucose levels ranged between 600 and 950 mg/dl, urinary glucose was 870–1000 mg/dl, ketones were detectable not more than once a week, and the weekly increase in body weight was 0.4–2%. In nondiabetic controls, blood glucose levels were 130–170 mg/dl, ketonuria was not present, and body weight increased 1.4–3.4% weekly. The diabetic–normoglycemic rats had blood glucose levels ranging between 40 and 210 mg/dl, only occasional glycosuria, no ketonuria, and a 1.4% weekly increase in body weight. During the diabetic phase, these animals had the same glucose and ketone levels and weight gain as the other diabetic rats.

Axonal transport

The distribution of total radioactivity along the sciatic system of diabetic rats at 28 d following intraspinal injection of labeled methionine is significantly different from that of controls (Fig. 1). Controls show a peak at 12 mm, corresponding to SCa (Fig. 2), and the bulk of the radioactivity migrating with SCb is contained in a segment 23–50 mm from the spinal cord (Figs. 1, 2). In the diabetic rats, most of the radioactivity is located in the proximal part of the system, and the radioactivity corresponding to SCb is significantly reduced (Fig. 1). This distribution indicates that slow transport is delayed in the sciatic system of diabetic rats. The delay cannot be due to a reduction of the total radioactivity transported, for the total radioactivity present in the sciatic system of diabetic rats ($908 \pm 216 \times 10^3$ cpm) was not significantly different from that of controls ($737 \pm 251 \times 10^3$ cpm).

The distribution along the sciatic system of the radioactivity present in individual bands in 1D-PAGE fluorograms shows a delay in the transport of all the polypeptides examined. The distribution of the labeled 145 kDa NF subunit shows a distinct peak at 15 mm from the cord in controls, whereas in diabetic rats the major peak is at 9 mm (Fig. 2). Identical changes in radioactivity distribution are also seen for the other 2 NF subunits (data not shown). The changes in tubulin transport are

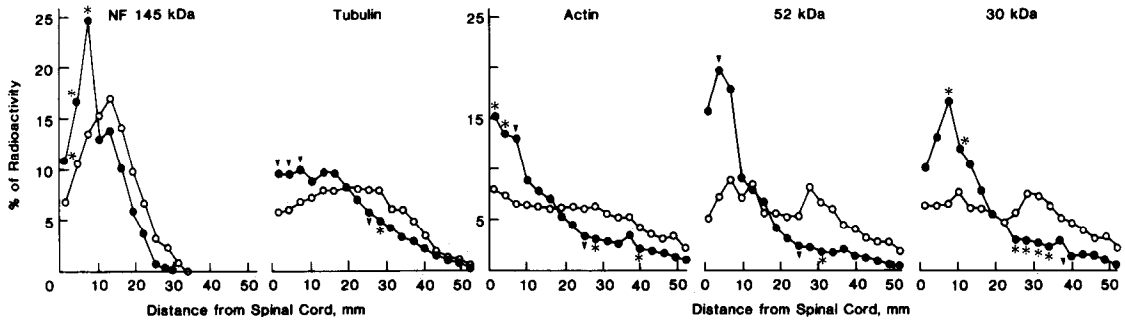

Figure 2. Distribution along the sciatic system of labeled 145 kDa neurofilament (NF) subunit, tubulin, actin, and the 52 and 30 kDa polypeptides. In diabetic rats (●), the peak of the radioactivity is significantly delayed for all polypeptides examined, as compared to controls (○). The peak of the labeled 145 kDa NF subunit, a representative polypeptide of SCa, is slightly delayed. Labeled tubulin and actin, which are normally transported in both SCa and SCb, as well as labeled 52 and 30 kDa polypeptides, which are considered to be constituents of SCb, are markedly delayed in diabetic rats. Unlike in the controls, no SCb peak can be detected. *$p < 0.05$; ▼, $p < 0.01$.

more complex. In normal peripheral axons, a part of tubulin is transported with SCa and another with SCb (Fig. 2). In diabetic rats, most of the labeled tubulin is located in the proximal segments, 3–21 mm from the cord, and there is a reduction in radioactivity in the nerve segments where tubulin transported with SCb is located (Fig. 2). This distribution suggests that, in diabetic rats, all the tubulin is transported at a single rate.

A more striking difference in radioactivity distribution is seen for the major polypeptides normally migrating with SCb (Fig. 2). In control rats, a labeled 52 kDa band, a major component of which is NSE (see below), and a labeled 30 kDa polypeptide tentatively identified as aldolase (see below) both peak at about 30 mm from the cord. In diabetic rats, however, the peak of these labeled components coincides with that of SCa at 9 mm from the spinal cord. A similar distribution is also seen for the other SCb components examined, including a 60 kDa polypeptide tentatively identified as pyruvate kinase, and a 20 kDa polypeptide (data not shown), as well as the part of tubulin transported with SCb. Transport rates, estimated using the 50th percentile, are decreased by 32% for the 145 kDa NF subunit and by 50–60% for the 30 and 52 kDa polypeptides (Table 1). Thus the transport of SCb polypeptides appears to be more severely impaired than that of SCa.

Very similar findings were obtained in the primary visual system. Twenty-five days following administration of ^{35}S-methionine, impairment of transport of NF subunits and tubulin is less severe than that of polypeptides transported with SCb (Fig. 3). Among the latter, actin and the 52 and 60 kDa polypeptides are more severely affected than the 30 kDa polypeptide. The distribution of radioactivity at 42 d after labeling shows that transport of the 145 kDa NF subunit, and, to a lesser extent, of tubulin, is slower but not blocked; the wave of these labeled polypeptides, which at 25 d was too proximal to be detected, has clearly moved into the system (Fig. 4). Similarly, the peak of the 30 kDa polypeptide has moved from the middle to the end of the system. The labeled 52 kDa polypeptides, however, are still mostly located in the proximal part of the system in diabetic rats, while in controls they have already reached the end of the system (Fig. 4). This distribution suggests that at least part of these polypeptides is transported very slowly or becomes stationary in the optic nerve. In diabetic–normoglycemic rats, all the labeled polypeptides examined appeared to be transported at a slightly faster rate than that of diabetes-resistant rats, but the differences were not statistically significant (Fig. 3).

Table 1. Transport rates of individual polypeptides in the sciatic system

	Transport rate (mm/28 d)[a]		Rate decrease (%)
	Control	Diabetes	
68 kDa NF	13.6 ± 1.0	9.5 ± 1.6*	30
145 kDa NF	13.4 ± 1.9	9.0 ± 1.2*	32
52 kDa	20.3 ± 3.6	8.9 ± 1.7**	56
30 kDa	23.2 ± 5.0	11.7 ± 2.8*	50

[a] Mean location (distance from spinal cord, in mm) of the 50th percentile of labeled polypeptides in the sciatic system 25 d following intraspinal injection of ^{35}S-methionine. Values expressed as means ± SD of data from 3 diabetic and 3 control rats.

* $p < 0.03$; ** $p < 0.008$.

Identification of polypeptides

The presence of labeled SCb polypeptides in the proximal region of the optic system of diabetic rats 25 d after labeling the retinal ganglion cells was confirmed by their location in fluorograms of 2D-PAGE and NEPHGE. In control rats, only SCa polypeptides are present in the proximal part of the optic system (Fig. 5*A*), while a distinct set of polypeptides transported with SCb is already in the distal part (Fig. 5*B*). In diabetic rats, labeled polypeptides of both SCa and SCb are in the proximal part of the system (Fig. 5*C*). The mobility in the gels of the SCb polypeptides in the diabetic rats was indistinguishable from that of controls.

Figure 5 also clearly shows the polypeptides present in the bands analyzed in 1D-PAGE. Labeled SCb polypeptides of 20, 30, and 52 kDa are well-resolved from the SCa polypeptides. There are 2 52 kDa polypeptides; the more acidic one was identified as NSE on immunoblots (Fig. 6). The other 52 kDa polypeptide is likely to be creatine kinase, which in the rat has a similar isoelectric point, but which is slightly larger in size than in guinea pig (Brady, 1982). The basic 60 and 30 kDa polypeptides have a mobility similar to those reported for pyruvate kinase and aldolase, respectively (Brady, 1982).

Morphometry

The distribution of axons as a function of axonal cross-sectional area (Fig. 7) in diabetic rats is significantly different from that of controls ($p < 0.0001$; Kolmogorov-Smirnov test). In diabetic

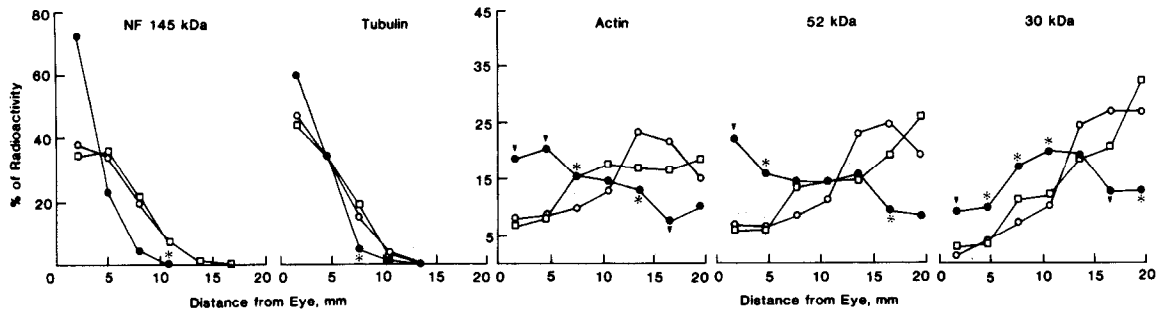

Figure 3. Distribution along the primary visual system of labeled 145 kDa NF subunit, tubulin, actin, and the 52 and 30 kDa polypeptides in diabetic (●), control (○), and diabetic–normoglycemic (□) rats 25 d following intraocular injection of ^{35}S-methionine. The distribution of labeled 145 kDa NF subunit and tubulin, which in the visual system migrate exclusively with SCa, is slightly but significantly retarded compared to control and diabetic–normoglycemic rats. More severely retarded is the transport of actin and the 52 and 30 kDa polypeptides. While in control and diabetic–normoglycemic rats the bulk of these labeled polypeptides has reached the distal segments, in diabetic rats it is still largely located proximally. The distribution of the radioactivity in diabetic–normoglycemic rats is not significantly different from that in controls. However, transport appears to be slightly but consistently faster in diabetic–normoglycemic rats. *$p < 0.05$; ▼, $p < 0.02$.

rats, axons are increased in size at the level of the spinal motor roots and decreased at the level of the tibial nerve. The mean cross-sectional area in proximal axons is 37.9 ± 3.8 in diabetic and 26.3 ± 5.5 in control rats ($p < 0.04$), corresponding to a 44% increase in the diabetic rats. In distal axons, the mean cross-sectional area is 10.4 ± 0.8 in diabetic and 17.0 ± 5.0 in controls ($p < 0.02$), corresponding to a 31% reduction in the diabetic rats.

Discussion

Diabetic BB rats spontaneously develop an insulin-dependent diabetes caused by a cell-mediated immune destruction of the pancreatic B cells (Like et al., 1979; Nakhooda et al., 1981a, b; Rossini et al., 1985). The onset of diabetes is abrupt, usually occurring between 2 and 4 months of age, and is characterized by hyperglycemia, ketoacidosis, polyuria, glycosuria, and ketoaciduria, which lead to death unless insulin is given (Marliss et al., 1981, 1982). Moreover, diabetic BB rats develop a progressive distal polyneuropathy with axonal degeneration in the late stages of diabetes (Mendell et al., 1981; Sima et al., 1983). Because of these and other features, diabetic BB rats are considered an excellent model of the human insulin-dependent diabetes (Nakhooda et al., 1977, 1978; Marliss et al., 1981, 1982; Mendell et al., 1981; Sima et al., 1983; Rossini et al., 1985).

The present study shows that slow axonal transport and axonal caliber are altered in this animal model of diabetes and suggests that the transport changes depend on the presence of hyperglycemia, since the changes were not observed in diabetic BB rats that were maintained normoglycemic with appropriate doses of insulin. Slow axonal transport is retarded in central and peripheral axons. Both SCa and SCb are affected, but SCb seems to be more so than SCa. The cause for the delay in transport of SCa and SCb has not been definitely established in the present study. Specifically, we did not determine whether the delay in SCa and SCb is due in part to a delay in transport initiation or solely to a decrease in transport rate, or whether a fraction of the transported proteins becomes stationary. However, in the sciatic system the delay in transport of NF proteins is likely to be due to the reduction of transport rate, in view of the changes in axonal caliber that we observed (see below). In the primary visual system, the analysis of transport at 2 different points in time shows that transport impairment is not due to a block at any point along the system. At 42 d after labeling, some of the labeled polypeptide constituents of SCb, such as actin and the 52 and 60 kDa polypeptides, are still located in the proximal half of the system (Fig. 4). This finding suggests that at least a fraction of these polypeptides moves at a much slower rate or becomes stationary. The distribution of other labeled polypeptides, such as the 145 kDa NF subunit and the 30 kDa polypeptide, is not consistent with the presence of a stationary component, but with a reduction of transport rate. Collectively, transport and morphometric data indicate that the delay in

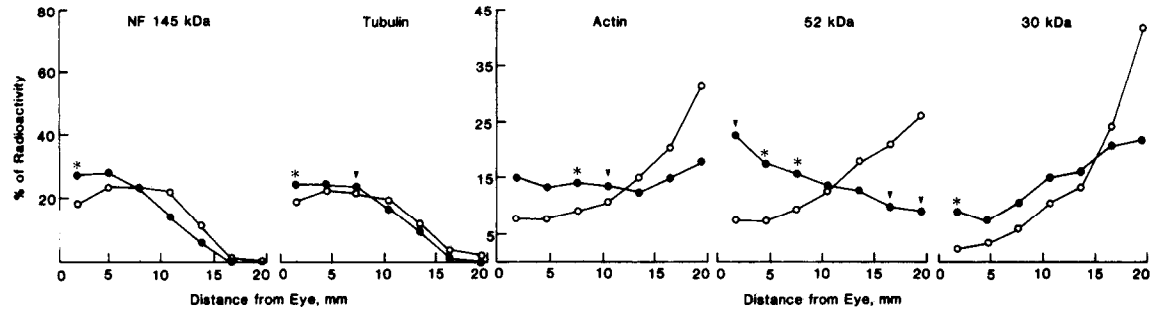

Figure 4. Distribution of the same labeled polypeptides shown in Figure 3 along the primary visual system of diabetic (●) and control (○) rats 42 d following administration of ^{35}S-methionine. In diabetic rats, transport of 145 kDa NF subunit and tubulin is still slightly retarded, but not blocked, as the peak of both labeled polypeptides has advanced in the optic nerve. In diabetic rats, a large fraction of labeled actin and of 52 kDa polypeptides is still in the proximal regions of the system, suggesting that the transport of these polypeptides is markedly slowed or that a fraction of them has become stationary. Transport of the 30 kDa polypeptides appears to be less affected. *$p < 0.05$; ▼, $p < 0.02$.

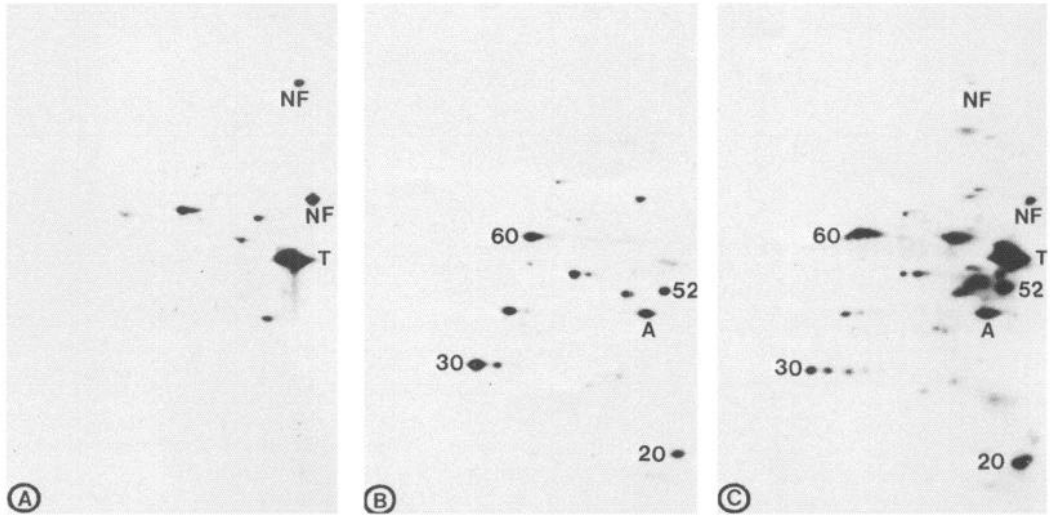

Figure 5. Fluorograms obtained from nonequilibrium pH gel electrophoresis (NEPHGE) of the optic system 25 d following intraocular administration of ^{35}S-methionine. The typical patterns of polypeptides transported with SCa and SCb are seen, respectively, in the proximal optic nerve (*A*) and distal optic tract (*B*) of a control rat. In contrast, the proximal optic nerve of a diabetic rat (*C*) contains labeled polypeptides of both SCa and SCb, confirming that transport of these polypeptides is delayed. *Numbers* indicate molecular weights (in kDa). *T*, tubulin; *A*, actin; *NF*, neurofilament subunits.

transport of SCa and SCb is due to a reduction of transport rate and that the transport of individual polypeptides is affected differently.

In addition to the transport changes, diabetic BB rats also showed distinct alterations of axonal caliber, which increased at the level of the motor roots and decreased at the level of the tibial nerve. The axonal atrophy in the tibial nerve is similar to that previously reported by Sima et al. (1983). The earlier detection of the atrophy in our animals, after approximately 3 months of diabetes as compared to 11 months in the previous study, may be explained by the higher blood glucose levels at which animals were maintained in our study (600–1000 mg/dl versus 300–350 mg/dl), which may have hastened the diabetic axonopathy. In the study by Sima et al. (1983), no increase in caliber of motor root axons was detected between 6 and 11 months of diabetes. However, in that study, axon and myelin sheaths were measured together, whereas we measured axons alone, a method obviously more accurate for detecting changes of axonal caliber.

The changes in slow transport and axonal caliber of the diabetic BB rats are virtually identical to those we recently reported in rats with SZ-induced diabetes (Table 2; Medori et al., 1985). The presence of the same changes in slow transport and axonal caliber in 2 different animal models of insulin-dependent diabetes argues that these changes are specifically and consistently associated with this type of diabetes and therefore may also be present in human insulin-dependent diabetes.

In a morphometric study of sciatic nerve axons from rats with SZ-induced diabetes, proximal and distal changes in axonal caliber were found to correlate with changes in the number of axonal NFs and microtubules (MT) (Medori et al., 1988). It has been shown that if the number of NF and MT inserted into the axonal transport remains constant, the number of NF and MT in a given segment of the axon is inversely related to their rate of transport in that axonal segment (Wujek et al., 1986; Monaco et al., 1988). Thus, changes of axonal caliber in SZ rats are likely to be secondary to the transport alteration. The enlargement of the proximal axons can be easily attributed to the slowing of the slow transport. It is more difficult to explain the distal dwindling in the absence of transport data in these axonal regions. Preliminary experiments with axonal models indicate that distal dwindling associated with proximal enlargement occurs when the slowing of the transport is relatively less severe in the distal than in the proximal regions of the axon (R. Medori and P. Gambetti, unpublished observations). Because of the similarity of transport and caliber changes between the 2 animal models, these conclusions are likely to apply also to the BB rat. Therefore, we propose that, in the early stages of the axonopathy associated with insulin-dependent diabetes, changes in axonal caliber are a result of an alteration of slow axonal transport.

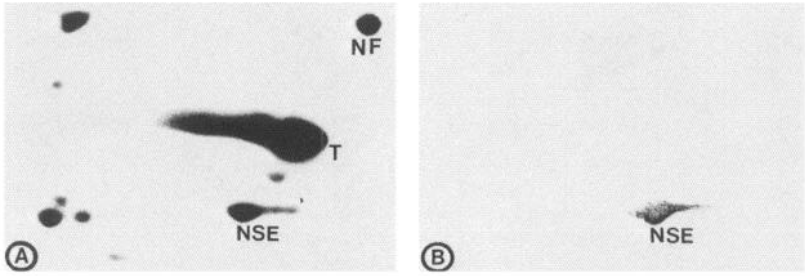

Figure 6. Identification of one of the labeled 52 kDa polypeptides in the proximal optic nerve of a diabetic rat. Autoradiogram (*A*) and immunoblot (*B*) obtained from a 2D-PAGE after transfer to nitrocellulose. The labeled 52 kDa polypeptide (*NSE*) in the autoradiogram is recognized by an antiserum to NSE in the immunoblot. *NF*, 68 kDa; *T*, tubulin.

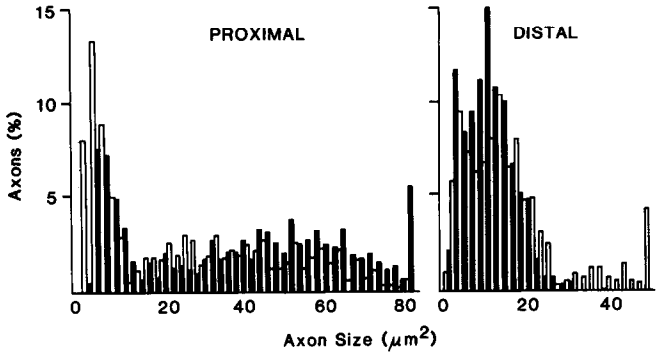

Figure 7. Histogram of percentage distribution of axons in diabetic (*solid bars*) and control (*open bars*) rats as a function of cross-sectional area. *Proximal:* Axons at the level of lumbar motor roots, 10–15 mm from the spinal cord (676 axons from control and 769 from diabetic rats). *Distal:* Axons at the level of the tibial nerve, 120–125 mm from the spinal cord (615 axons each from control and diabetic rats). Nerves from diabetic animals contain a higher number of larger axons proximally, and of smaller axons distally. Histograms from diabetic rats are significantly different from controls at both distal and proximal levels ($p < 0.0001$).

Table 2. Changes in axonal transport and axonal caliber in the sciatic system of BB diabetic rats and rats with streptozotocin (SZ)-induced diabetes[a]

	BB (%)	SZ (%)
Decrease in transport rate[b]		
68 kDa	30	22
NSE	56	40
30 kDa	50	37
Changes in axonal cross-sectional area		
Motor roots[c]	+44	+39
Tibial nerve[d]	−31	−37

[a] Data obtained from Medori et al. (1985).
[b] Determined from the location of the 50th percentile (Hoffman et al., 1983).
[c] Distance from the spinal cord, 10–15 mm.
[d] Distance from the spinal cord, 120–125 mm.

In a recent study of slow axonal transport in diabetic mutant mice, a model of non-insulin-dependent diabetes, it was concluded that transport of SCa is retarded, whereas that of SCb is unchanged (Vitadello et al., 1985). Yet the 2 SCb polypeptides analyzed, actin, and tubulin which migrates in part with SCb, were found to be transported at a slower rate. Thus, both SCa and SCb might also be affected in non-insulin-dependent diabetes.

The consistent presence of the same changes in slow transport and axonal size in experimental models of diabetes raises questions concerning the mechanism responsible for these changes and the possible role of these changes in the pathogenesis of the axonal degeneration that characterizes the later stages of human and animal diabetic neuropathy (Brown and Ashbury, 1984; Thomas and Eliasson, 1984).

Decreased neuronal protein synthesis, leading to a diminution of proteins transported in the axon, and multiple endoneurial infarcts, 2 pathogenic mechanisms proposed for diabetic neuropathy, cannot have caused the transport impairment that we have observed in SZ and BB rats (Chihara, 1981; Chihara et al., 1982; Dyck et al., 1986a, b; Johnson et al., 1986). Unlike previous investigators, we did not find that the total radioactivity of proteins transported in the sciatic system in these 2 animal models was significantly different from that of controls (Chihara, 1981; Chihara et al., 1982; Medori et al., 1985). Moreover, we analyzed distribution and not amount of transported radioactivity. Although microinfarcts have occasionally been reported in the motor roots of BB rats after 11 months of diabetes (Sima and Thibert, 1982), neither infarcts nor axonal degeneration has been observed by ourselves and others (Nakhooda et al., 1978; Mendell et al., 1981; Sima et al., 1983) in SZ and BB rats at earlier stages of diabetes. Moreover, axonal degeneration due to infarct, presumably followed by regeneration, would lead to changes of slow transport quite different from those that we have observed (Hoffman et al., 1985).

Endoneurial hypoxia, or a decreased activity of Na^+,K^+-ATPase, might alter axonal transport by impairing basic mechanisms of energy supply. However, under these conditions, one would expect that transport of polypeptides that migrate with the same transport component and presumably share the same transport mechanisms is affected to the same degree, whereas our findings are more consistent with a selective transport impairment of individual polypeptides. Moreover, it has recently been shown that anoxia similar to that supposedly present in peripheral nerve during diabetes has no effect in slow axonal transport (Nagata et al., 1987). A selective transport impairment is best explained by nonenzymatic glycosylation, a common complication of diabetes that results from the direct chemical reaction between glucose and amino groups of proteins (Kennedy and Baynes, 1984; Panush Cohen, 1986). A variety of proteins, including tubulin, have been reported to be glycosylated in experimental and human diabetes (Kennedy and Baynes, 1984; Panush Cohen, 1986). One may postulate that glycosylation interferes with the transport of individual proteins to a degree directly related to the extent of glycosylation. Williams et al. (1982) have reported that an increased amount of covalently bound reducing sugars is present in tubulin isolated from the brains of rats with SZ-induced diabetes and in high-molecular-weight material that did not enter the gel. Although individual proteins were not purified and reduced sugars were not searched in our study, we did not detect any change of molecular weight or isoelectric point, nor an increase in high-molecular-weight material not entering the gels. Further studies are obviously needed to assess whether nonenzymatic glycosylation plays a role in the impairment of the slow axonal transport associated with diabetes.

Since it is likely that the transport changes of SZ and BB rats also occur in human diabetic neuropathy, it is important to know whether these changes could be responsible for the axonal degeneration that characterizes the more advanced stages of this neuropathy. The axon receives almost all of its constituents (Grafstein and Forman, 1980; Lasek et al., 1984) from the cell body through axonal transport, and it is obvious that an interruption of the transport results in axonal degeneration. However, the role that the individual components of the transport play in maintaining the structural integrity of the axon is not clear (Thomas and Eliasson, 1984). Experimental studies indicate that a severe impairment of NF transport, a major constituent of SCa, leads to alterations of axonal caliber but not to degeneration of the axon (Bizzi et al., 1984; Hoffman et al., 1985; Medori et al., 1985; Monaco et al., 1985; Gambetti et al., 1986; Wujek at al., 1986). Unlike SCa, which comprises only cytoskeletal proteins, SCb carries a large number of enzymes

(Lasek et al., 1984). NSE, PK, CPK, and aldolase, all enzymes involved in the generation of energy from glycolysis, as well as calmodulin, a protein implicated in the regulation of a wide variety of enzyme activities and cell processes, have been shown to be transported with SCb (Cheung, 1980; Brady and Lasek, 1981; Brady et al., 1981). Among the SCb polypeptides whose transport is severely affected we have identified NSE. Although the identity of the other polypeptides was not determined, polypeptides of 60 and 30 kDa with similar isoelectric points, migrating with SCb in the guinea pig optic system, have been identified as PK and aldolase (Brady, 1982). Thus, energy supply from the glycolytic pathway, which is required to maintain function, is likely to be impaired in the diabetic axon (Sabri and Ochs, 1972). Other metabolic pathways depending on SCb polypeptides may also be altered. Another constituent of the SCb complex in peripheral nerve that is affected in experimental diabetes is tubulin. Although there are differences between the tubulin transported with SCa and that with SCb (Sabri and Ochs, 1972; Brady, 1982; Morris and Lasek, 1982; Brady et al., 1984; Tashiro et al., 1984; Brady and Black, 1986; Sahenk and Brady, 1987) both are largely in polymerized form; most of SCa MT are thought to be cold and Ca^{2+}-stable, whereas SCb MT are in dynamic equilibrium with tubulin (Brady et al., 1984; Brady and Black, 1986; Sahenk and Brady, 1987). A sustained impairment of tubulin transport may result in a marked diminution in the number of MTs in the distal axon, which may in turn alter fast anterograde and retrograde axonal transport (Snapp et al., 1986). Thus the transport changes that we have observed in experimental insulin-dependent diabetes could lead to axonal degeneration. Future studies should determine whether axonal degeneration of human diabetic neuropathy is indeed due to impairment of the slow transport.

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