Experimental diabetic neuropathy: Impairment of slow transport with changes in axon cross-sectional area
(dia:axonal transport/streptozotocin/axonal caliber)

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ABSTRACT Analysis of slow axonal transport in the sciatic and primary visual systems of rats with streptozotocin-induced diabetes of 4-6 weeks duration showed impairment of the transport of neurofilament subunits, tubulin, actin, and a 30- and a 60-kDa polypeptide in both systems. The degree of impairment was not uniform. Transport of polypeptide constituents of the slow component b, such as the 30- and 60-kDa polypeptides, appeared to be more severely affected than the transport of constituents of the slow component a, such as neurofilaments. Morphometric analysis of sciatic axons revealed a proximal increase and a distal decrease of axonal cross-sectional area. It is proposed that impairment of axoplasmic transport and changes of axonal size are related. Transport impairment results in a larger number of neurofilaments, microtubules, and other polypeptides in the proximal region of the axon, which increases in size, whereas fewer neurofilaments, microtubules, and other polypeptides reach the distal axons that show a size decrease. Such changes in axonal transport and area are likely to occur in other diabetic animal models and in human diabetes.

Peripheral nerve dysfunction is a common complication of human diabetes mellitus (1, 2). Clinical symptoms of peripheral neuropathy are present in approximately 25% of diabetic individuals, while nearly all diabetics have a reduction of nerve conduction velocity (3–7). Peripheral nerve pathology in diabetic polyneuropathy is characterized by axonal atrophy and degeneration. Segmental demyelination and hypertrophy and proliferation of Schwann cells are occasionally observed, but these changes are thought to be secondary to the axonal involvement (8, 9). Several mechanisms have been considered to explain the pathogenesis of the diabetic polyneuropathy (10–15); one of the most intriguing proposes that axonal atrophy and degeneration are secondary to an impairment of axonal transport, which is in turn related to the metabolic defects of diabetes (16).

Studies of slow axonal transport in experimental and congenital animal models of diabetes have been contradictory, showing either impairment or no change (16–21). In those studies, however, only the total radioactivity migrating along the axon with the components a (SCa) and b (SCb) of the slow transport was examined. We analyzed transport of individual polypeptides in central and peripheral axonal pathways and carried out a morphometric study of peripheral axons from rats with streptozotocin (SZ)-induced diabetes. Evidence was obtained that transport of actin, neurofilament (NF) subunits, tubulin, and 30- and 60-kDa polypeptides is impaired in both central and peripheral axons of diabetic animals. This transport impairment is accompanied by a proximal increase and a distal decrease of the axonal caliber.

MATERIALS AND METHODS

Induction of Diabetes. Male Wistar rats (19 weeks old) were injected intraperitoneally with 65 mg of SZ (Upjohn) per kg of body weight in 1 ml of 10 mM sodium citrate buffer (pH 4.5), after 8 hr of fasting (22–25). Age- and weight-matched control rats received citrate buffer in identical amount and pH. Nonfasting blood glucose levels (Beckman glucose analyzer 2) were measured 48 hr after intraperitoneal injections, then once a week and immediately prior to sacrifice. Animals were weighed before the injections and weekly thereafter.

Axonal Transport. Rats with 4- to 6-week diabetes and controls received [35S]methionine in sterile saline by intraspinal (750 μCi in 3 μl; 1 μCi = 37 GBq) or intraocular (500 μCi in 4.5 μl) injections as previously described (26, 27). Animals were sacrificed 25 days after labeling by intra-aortic perfusion of saline, and the primary visual and sciatic systems were dissected out. Optic nerves and tracts were cut into 3-mm segments; the chiasma, which measured approximately 2 mm, and the superior colliculus were also obtained. The sciatic system from the spinal motor roots to the tibial nerve was cut in 3-mm segments. Individual nerve segments were processed for electrophoresis and fluorography as previously described (27, 28). Before electrophoresis, the total radioactivity of each segment was determined. The distribution of the radioactivity related to selected polypeptides of the slow transport was assessed either by determining the radioactivity in the appropriate gel bands or by scanning the fluorogram (28–30). Polypeptides selected for this analysis were the 145- and 68-kDa NF subunits, the 55- and 43-kDa polypeptides that constitute tubulin and actin, respectively, and 60- and 30-kDa polypeptides. The two most proximal motor root segments were used only for the analysis of NF because local labeling of these segments affected the radioactivity of other polypeptides (29, 30). The transport rates of the 68-kDa subunit and of the 60- and 30-kDa polypeptides were determined by expressing the cumulative percentile of radioactivity as a function of the distance along the nerve. The 50th percentile was taken as a measure of the distance traveled by the wave of radioactivity related to these polypeptides. The two-tail unpaired Student t test was used for statistical analyses. Data in Figs. 1, 2, and 4 are based on three control and three diabetic rats.

Axonal Morphometry. Three male Wistar rats with 4–6 weeks of SZ-induced diabetes and three controls were

Abbreviations: SZ, streptozotocin; NF, neurofilament(s); SCa, slow component a; SCb, slow component b; MT, microtubule(s).

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†The slow transport has two components, which travel at different rates and which are referred to as slow component a (SCa) and slow component b (SCb). The slower-moving SCa carries neurofilament (NF) subunits and tubulin, whereas the faster SCb conveys several proteins, including enzymes of intermediary metabolism and cytoskeletal proteins (32).
perfused through the aorta with 0.2 M sodium cacodylate buffer (pH 7.4; 400 millimolar) for 40 sec followed by 5% (wt/vol) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4; 700 millimolar) for 20 min. Right and left sciatic systems were dissected out, fixed overnight, and cut into 5-mm segments. Segments from motor roots (10-20 mm from spinal cord) and from spinal (35-45 mm from spinal cord) and tibial nerves (110-120 mm from the spinal cord) were treated with osmium tetroxide, dehydrated, and embedded in Spurr resin. The cross-sectional area of each axon and percentage distribution as a function of size were determined on thick sections by using a Leitz microscope equipped with a video camera interfaced to a Bioquant morphometric system digitizer (31). One section from each level of right and left sciatic systems was examined from each animal. In each section, 380 randomly selected axons, representing approximately 32% of the total, were examined.

![Graph](image)

**Fig. 1.** Distribution of total $[^{35}S]$methionine radioactivity migrating in the sciatic system with the slow transport in diabetic (■) and control (○) rats. While in controls the peaks of SCa and SCb are distinct, in diabetic rats these peaks cannot be detected and the radioactivity is distributed as a proximodistal gradient, with a larger amount of radioactivity still located in the proximal part of the sciatic system. Here and in Figs. 2 and 4, ▼ indicates $P < 0.05$ that diabetic result is same as control, and * indicates $P < 0.03-0.001$.

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### RESULTS

**Diabetes.** Serum glucose concentrations ranged between 450 and 800 mg/dl in diabetic animals and 120 and 160 mg/dl in controls. Body weight decreased an average of 11% per week during the first 3 weeks of diabetes and remained stable afterward, while it increased 4-6% weekly in controls.

**Axonal Transport.** The distribution of the total radioactivity migrating in the sciatic system with the slow axonal transport in diabetic animals was markedly different from that of controls (Fig. 1). In diabetic animals a larger portion of radioactivity was located in the most proximal segments, 9-15 mm from the spinal cord, whereas less radioactivity was present distally at 39-48 mm, where SCb had migrated in controls. As a result of these changes, the radioactivity in diabetic rats was distributed as a proximodistal gradient and did not show the two distinct peaks corresponding to SCa and SCb that were present in controls. This distribution indicates that in diabetic rats the rate of slow transport is decreased.

Analysis of the radioactivity migrating with the individual polypeptides revealed that transport of the 68- and 145-kDa NF subunits, tubulin, actin, and 60- and 30-kDa polypeptides was significantly delayed, although to different extents (Fig. 2). The transport impairment was especially striking for the 60-kDa polypeptide. The bulk of the radioactivity related to this polypeptide, which is normally a prominent constituent of SCb, codistributed with SCa (Fig. 2B). The transport rate, as determined by the location of the 50th percentile of the migrating radioactivity, was decreased by 40% and 37% for

![Graph](image)

**Table 1. Decrease of transport rate in diabetic rats**

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Control</th>
<th>Diabetes</th>
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<tbody>
<tr>
<td>68-kDa NF</td>
<td>12.1 ± 1.5</td>
<td>9.4 ± 0.7*</td>
</tr>
<tr>
<td>60-kDa</td>
<td>35.1 ± 6.0</td>
<td>21.3 ± 1.2</td>
</tr>
<tr>
<td>30-kDa</td>
<td>30.3 ± 2.6</td>
<td>19.0 ± 1.4*</td>
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*Mean location (mm from spinal cord) of 50th percentile of radioactivity migrating with the individual polypeptides in the sciatic system 25 days after labeling of sciatic motor neurons. Results are means (±SD) of values for three diabetic and three control animals.

1$P < 0.05$.

$*P < 0.005$. 

**Transport.** The distribution of the total radioactivity migrating in the sciatic system with the slow axonal transport in diabetic animals was markedly different from that of controls (Fig. 1). In diabetic animals a larger portion of radioactivity was located in the most proximal segments, 9-15 mm from the spinal cord, whereas less radioactivity was present distally at 39-48 mm, where SCb had migrated in controls. As a result of these changes, the radioactivity in diabetic rats was distributed as a proximodistal gradient and did not show the two distinct peaks corresponding to SCa and SCb that were present in controls. This distribution indicates that in diabetic rats the rate of slow transport is decreased.

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the 60- and 30-kDa polypeptides, respectively, while the reduction for the NF was 22% (Table 1). Transport of actin and tubulin appeared to be affected to a degree intermediate between that of NF and that of the 60-kDa polypeptide. The component of tubulin normally migrating with SCb (32) was not detectable, and all the tubulin appeared to distribute with SCa.

In the visual system, analysis of transport of individual polypeptides showed a moderate degree of impairment in the transport of NF and tubulin, whereas the impairment was much more severe for actin and for the 60- and 30-kDa polypeptides, which are components of SCb also in the optic system (Figs. 3 and 4). While in controls most of the radioactivity related to these polypeptides had migrated to the optic tract, in the diabetic animals it was almost evenly distributed along the entire system (Fig. 4). Therefore, the polypeptides affected were the same in both visual and sciatic systems. In both systems the polypeptides of SCb appear to be more severely affected than those of SCa.

**Axonal Morphometry.** The size of axons in the sciatic system of diabetic rats was increased proximally, unchanged in the intermediate regions, and decreased distally (Table 2; Fig. 5). The mean cross-sectional area of the proximal axons was 24.5 \( \mu m^2 \) in diabetic animals and 17.6 \( \mu m^2 \) in controls, which corresponds to an increase of approximately 40%. In the intermediate region mean axonal size was not significantly different in diabetic and control animals, whereas distally it was 6.7 \( \mu m^2 \) in diabetic and 10.4 \( \mu m^2 \) in controls, corresponding to a reduction of approximately 36% in size of diabetic axons (Table 2). Histograms of percent distribution of axons as a function of size showed that in diabetic animals the increase in size proximally and the decrease distally affected both small and large populations of axons normally present in the sciatic system (Fig. 5).

**DISCUSSION**

Administration of SZ causes a selective destruction of \( \beta \) cells of the pancreas, which results in decreased production of insulin and high levels of serum glucose (22, 23). Rats given SZ are models of human type I diabetes (23, 33); they display reduced nerve conduction velocity and prolonged preservation of nerve function during ischemia, two common changes in diabetic patients (34, 35). These changes are secondary to the induced diabetes and not to a direct effect of SZ on peripheral nerve, as they can be reversed by insulin treatment (10, 21).

The present study shows that slow axonal transport is impaired in animals with SZ-induced diabetes. The separation between SCa and the faster-moving SCb, normally observed in the sciatic and visual systems 25 days after administration of radioactive precursors, was markedly reduced. Transport of all individually analyzed polypeptides was affected but not uniformly. The transport rate was decreased more for the 60-kDa polypeptide than for the NF subunits, and transport of tubulin appeared to be impaired more severely than that of actin. The bulk of the radioactivity related to the 60- and 30-kDa polypeptides and probably that related to the rapidly moving component of tubulin, which normally are constituents of SCb (32), were found to codistribute with SCa. Transport of the 60- and 30-kDa polypeptides was similarly affected in the optic system. Proteins transported with SCb, therefore, appear to be more severely affected than those transported with SCa.
These changes of slow axonal transport in diabetic animals are independent of possible changes in neuronal protein synthesis, since we measured the distribution rather than the amount of the labeled proteins transported in the axon. Actually, we found higher levels of radioactivity in nerves from diabetic animals than in those from controls. Therefore, our findings do not support previous reports that neuronal protein synthesis is decreased in animals with SZ-induced diabetes (36).

In previous studies on slow axonal transport in rats with SZ-induced diabetes, Jakobsen and Sidenius (19) showed an impairment of SCa in the sensory system of rats with 3-week diabetes, whereas no significant changes were detected by Biscoy (20) in the same system after 2–6 weeks of diabetes.

The present study resolves this controversy and demonstrates that in diabetes there is a similar impairment of axonal transport in both central and peripheral axons. The finding that diabetes has an effect on transport in central axons is not surprising, since there is a large body of evidence that in diabetes both metabolism and functions of the central nervous system are affected (37–39). The paucity of clinical signs of central nervous system involvement in diabetes (40, 41) may be explained on the basis of a preferential vulnerability of peripheral over central axons that is also observed in certain toxic conditions (27). Lack of overt clinical symptoms does not rule out the occurrence of subtle functional impairment. The present finding of a significant impairment of slow axonal transport in central axons emphasizes the involvement of the central nervous system in diabetes.

The nature of the 60- and 30-kDa polypeptides, the most severely affected of the polypeptides we have individually examined in diabetic rats, remains to be determined. Polypeptides of similar size migrating with SCb in guinea pig optic systems have been identified as pyruvate kinase and aldolase (42). A preferential impairment in transport of enzymes of intermediary metabolism might play an important role in the pathogenesis of diabetic polyneuropathy.

Another finding of the present study is that the cross-sectional area of the sciatic axons in diabetic animals is increased in the proximal segments, normal in the intermediate region, and decreased distally (see Fig. 6). Axonal size in chemical and genetic animal models of diabetes has been the subject of numerous studies, which consistently show a decrease of the cross-sectional area in distal segments of peripheral axons (43–47). The present study confirms this finding. In addition, our data establish that in chemical diabetes axons are increased in size proximally. Further morphometric analysis revealed that there is an identical linear relation between axonal size and number of NF in diabetic and control animals at proximal and distal levels (unpublished results); thus, in axons of equal size, NF densities are the same in diabetic rats and controls. The correlation between axon size and number of NF has been well documented (48). Since in diabetic rats the mean axon size was increased proximally and decreased distally, the total number of NF present in the proximal region of the sciatic system was higher, and that present in the distal region was lower, than that of controls. Therefore, the observed changes of axon caliber (Fig. 6) represent the adjustment of the axon to a proximal increase and a distal decrease of the total number of NF and perhaps MT and of other axonal organelles. In our model the unequal longitudinal distribution of NF and perhaps of MT and of other axonal organelles might result either from a gradient of impairment—i.e., a more severe transport impairment in the proximal than in the distal regions of the axon—or from a partial blockade of transport with retention of some of the NF, MT, and other structures in the proximal axons.

We have conducted similar transport and morphometric studies in primary visual and sciatic system of bb rats with genetic insulin-dependent diabetes (49, 50). Preliminary results show changes similar to those reported above in rats with SZ-induced diabetes (unpublished data). Moreover, preliminary data from another laboratory indicate that transport of NF and perhaps other cytoskeletal polypeptides is impaired in the db/db mouse, a genetic model of non-insulin-dependent diabetes (51). We propose that impairment of slow transport with secondary increase of axon area proximally and decrease distally is a feature common to all forms of animal diabetes and may also occur in human diabetes.

In spite of some superficial similarities, the axonopathy associated with SZ-induced diabetes is different from the proximal giant axonal neuropathies. This group of conditions includes neuropathies characterized by focal impairment or block of NF transport and massive but mostly focal increase in area of proximal axons with reduction in size distally (52, 53). Experimental neuropathies such as those induced by aluminum (29) and β,β'-iminodipropionitrile (26, 54, 55) and
probably human diseases such as amyotrophic lateral sclerosis (28, 56) are of this type.

In SZ-induced diabetes the impairment of axonal transport is not selective for NF but affects several other polypeptide components of both SCa and SCb. Moreover, the impairment of axonal transport and the proximal axonal enlargements are not focal, but they appear to involve, in a fairly uniform way, a large portion of the axon. Transport and morphologic changes of the axon associated with SZ-induced diabetes therefore appear different from those described in previous axonopathies.

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