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Research Article

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Decreased Axonal Transport of Structural Proteins in Streptozotocin Diabetic Rats

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ABSTRACT We have examined the various axonal transport rates in sciatic nerve of streptozotocin diabetic rats 3 h and 10, 25, and 50 d after the injection of tritiated proline into the fifth lumbar dorsal root ganglion. Proline-labeled proteins conveyed by the slow transport system were advanced more slowly in diabetic rats. No compensation for this delay took place in terms of protein synthesis, half-life, or transported amount. The decreased deliverance of slowly transported proteins (structural proteins) to the axons may well account for the reduced axon calibre shown in earlier reports. A hypothesis is proposed suggesting that the primary event in the development of neurological abnormalities in diabetes is an impairment of the retrograde axonal transport, secondarily leading to the abnormality of the anterograde transport of structural proteins.

INTRODUCTION

Streptozotocin diabetes in rats is associated with reduced myelinated nerve fibre caliber (1–3). The axon calibre increases in controls during the experimental period, whereas it decreases in diabetic rats despite normal nerve internodal length and normal skeletal length (1, 3).

The structural abnormality of the axon can be demonstrated after a few weeks' duration of the diabetic state and can be prevented by insulin treatment (1). It is, therefore, the result of the metabolic derangement, and not of long-term diabetic changes of intraneural vessels or of a toxic effect of streptozotocin itself.

The axon does not synthesize its own proteins but is dependent on supply from the nerve cell body (4–6). Newly synthesized proteins are conveyed to the axon and its terminals by the axonal transport system. Consequently, the axonal shrinkage and lack of "maturation" in diabetic rats might be the result of an abnormality of this transport.

Glycoproteins, phospholipids, and constituents of synaptic and neurosecretory vesicles are transported in the fast system at a velocity of 400 mm/d (6–8), whereas a slower transport of structural proteins, i.e., tubulin and neurofilaments, responsible for the outgrowth and renewal of the axonal mass, moves 1 mm/d (4–10). Recently, actin and other soluble proteins different from the structural proteins have been identified in a group moving at a velocity of 3–4 mm/d (11, 12). The group of structural proteins in the transport system are now termed slow component a (SCa)[1] and the recently discovered group slow component b (SCb) (12).

In the present experiments, we have examined both components of the slow axonal transport in rats of the same colony and age, and with a similar degree of diabetes as those used in the experiment demonstrating the structural axon abnormality (1–3).

METHODS

Diabetes was induced in male Wistar rats (Institute of Anatomy, Aarhus C, Denmark) by intravenous injection of 40 mg streptozotocin/kg body wt. Weight and age-matched animals were kept as controls. Blood glucose was measured in the diabetic group with an Ames reflectance meter (Ames Company, Inc., Slough, England) at start and end of the axonal transport experiment. Only rats having a blood glucose value > 250 mg/100 ml at both measurements were included in the study.

At the end of the experiment, all rats were 28 wk old and the diabetic state had persisted for at least 4 wk. This is similar to our previous experiments, as is the chosen dose of streptozotocin (3, 13–15).

Axonal transport experiments were carried out in the following groups.

3 h of fast axonal transport. To estimate the half-life of labeled proteins synthesized in the dorsal root ganglion and the fast transport velocity, precursors were injected 3 h before the transport was stopped.

Diabetes was induced in 24-wk-old rats weighing between 360 and 475 g, and the transport experiment was performed 4 wk later.

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[1] *Abbreviations used in this paper*: SCa, slow component a; SCb, slow combonent b; 2p, two-tailed *t* test.

10 d of slow axonal transport. This group allows an estimate of the transport velocity of SCb.

Rats 24 wk of age weighing between 305 and 465 g were used. The axonal transport experiment was started 18 d after the injection of streptozotocin and stopped after a further period of 10 d.

25 d of slow axonal transport. To estimate the SCa, rats were examined in this and the 50-d group described below.

At the age of 24 wk, rats weighing between 350 and 425 g were rendered diabetic; 3 d later the axonal transport experiment started.

50 d of slow axonal transport. Rats 20.5 wk of age weighing between 320 and 400 g were injected with streptozotocin. On the following day, precursor injection was performed. 50 d later the transport experiment was stopped.

Operative procedure, application of precursor, and counting of activity. Labeled proline ([L-2,3,4,5-^{3}H]proline, 117 Ci/mmol; Amersham, Buckinghamshire, England) was used as marker for the axonal transport of protein. The precursor was concentrated by freeze-drying and dissolved in a buffered salt solution to give a final concentration of 8–10 μCi/μl.

The animals were anesthetized by sodium pentobarbital, and the fifth lumbar dorsal root ganglion on the right side was exposed by a partial laminectomy. A glass micropipette with an external tip diameter of 40–50 μm was led into the ganglion, and 0.5 mm below the surface 1 μl of the precursor solution was delivered under air pressure. (For the 3-h experiment the fifth lumbar dorsal root ganglion on both sides was exposed by total laminectomy and injected.) In all experiments, body temperature was maintained constant during anesthesia by placing the rats on an electrically heated pad.

At the end of the experiments, the animals were reanesthetized with ether and killed by removal of the heart. The fifth lumbar ganglia and the sciatic nerves were dissected out, and axonal transport was stopped by cooling the nerve on a copperblock (−70°C).

The ganglion was separated and the rest of the nerve was cut into 3-mm segments that were placed overnight in numbered vials containing 1 ml ice-cold 10% Trichloroacetic acid. After wash in fresh Trichloroacetic acid for 1 h, tissues were dissolved in 150 μl Lumasolve (Lumac, Amsterdam, Holland) and subsequently counted for 10 min in 4 ml Lipoluma (a xylol scintillation fluid; Lumac). Counts were corrected for quenching and background and expressed as disintegrations per minute.

Calculations. In 2 of the 65 animals in whom axonal transport experiments were carried out, the total amount of activity was very low and no distinct front of activity appeared in the nerve, presumably as a result of an unsuccessful precursor injection. The two animals were excluded.

In the 10-d and 3-h groups, the position of the wavefront was the intercept between a line drawn through the wavefront and a line through the background activity ahead of the front (Fig. 1) (13). In the 50- and 25-d groups, a bell-shaped wave of activity could be seen moving distally with time (Fig. 2). The velocity of SCa was calculated partly as the mean velocity of all activity under the bell-shaped wave and partly as the velocity of the wave peak. The position of the wave was determined by the formula:

$$x_{mean} = \frac{A_1x_1 + A_2x_2 + \cdots + A_nx_n}{A_1 + A_2 + \cdots + A_n}$$

for the mean velocity. A_1, amount of activity in segment 1; x_1, distance between ganglion and segment 1; segment 1, first segment under the bell-shaped wave; segment n, last segment under the bell-shaped curve (Fig. 2). By applying the same formula on the four or five neighbouring segments with highest activity, the distance for determining the peak velocity was found. It was not possible to perform these calculations on the 3-h and the 10-d groups because the activity in the wave front could not be demarcated proximally. The transport velocity was calculated as the distance of the wavefront from the dorsal root ganglion divided by the number of days passed for the slow transport experiments. In the 3-h experiment, the velocity was calculated as the distance of the wavefront, divided by the time elapsed in hours minus the lag time from injection of precursors until start of fast transport as obtained in our previous studies (13).

The amount of protein in SCa after 25 and 50 d was expressed as the total activity below the bell-shaped wave relative to ganglion activity (Fig. 2).

The amount of protein activity in the ganglion was expressed as a function of time elapsed after precursor injection in all the groups of study. From the values for the 3-h group, the amount destined for the slow transport estimated from the 25- and 50-d groups was subtracted. The parameters for the regression line with time as the independent variable and the natural logarithm of the activity in the ganglion as the dependent variable were calculated.

The regression lines for the control and diabetic group were compared by the two-tailed F test of variance (16).

All other differences were examined for statistical significance with the two-tailed *t* test (17). Values are given as mean±CV (coefficient of variation, SD/mean) unless indicated otherwise.

RESULTS

Body weight for all animals and blood glucose values for the diabetic rats during the period for the axonal transport experiment are shown in Table I. All diabetics lost weight and had a considerable elevation in blood glucose.

In the sciatic nerve of each animal included in the various groups of the study, a distinct wavefront of protein activity was found that allowed calculation of the transport velocity.

Transport velocity. No difference was obtained between controls and diabetics in the group used for examination of the fast axonal transport velocity 3 h after the injection of tritiated proline into the dorsal root ganglion. The mean velocity was 15.5±0.081 and 15.5±0.061 mm/h, respectively.

SCb was examined 10 d after the injection of labeled precursor. The mean curves of activity along the nerve for the control group and the diabetic group are shown in Fig. 1. No differences between the two groups appear. The mean transport velocity in the control group was 4.53±0.059 and 4.43±0.23 mm/d in the diabetic group.

SCa, the carrier of structural proteins, was examined after 25 and 50 d. The mean curves after 25 d of labeled protein activity as a function of the distance from the ganglion are shown in Fig. 2 for the control group and the diabetic group. The mean velocity in controls and diabetics was 1.03±0.076 and 0.88±0.12 mm/d, respectively, and the difference amounting to 15% is statistically significant/(two-tailed *t* test [2p]) = 5.8×10^{-3}).

TABLE I

*Body Weight at the Start and the End of the Axonal Transport Experiment in Control and Diabetic Groups as well as Blood Glucose Values at the Same Points of Time in the Diabetic Groups Only**

Experimental group		Body weight		Blood glucose	
		At the time of tracer injection	At the end of the experiment	At the time of tracer injection	At the end of the experiment
		g		*mg/100 ml*	
3 h	Control (9)‡	—	429±40	—	—
	Diabetes (8)	—	304±29	—	358±32
10 d	Control (7)	418±45	403±47	—	—
	Diabetes (8)	341±41	303±40	411±35	388±56
25 d	Control (8)	392±29	422±22	—	—
	Diabetes (7)	376±26	274±23	389±46	444±58
50 d	Control (9)	353±38	399±34	—	—
	Diabetes (7)	363±35	238±30	364±48	421±51

* Values are mean±SD.

‡ Number in parentheses indicates number in experimental group.

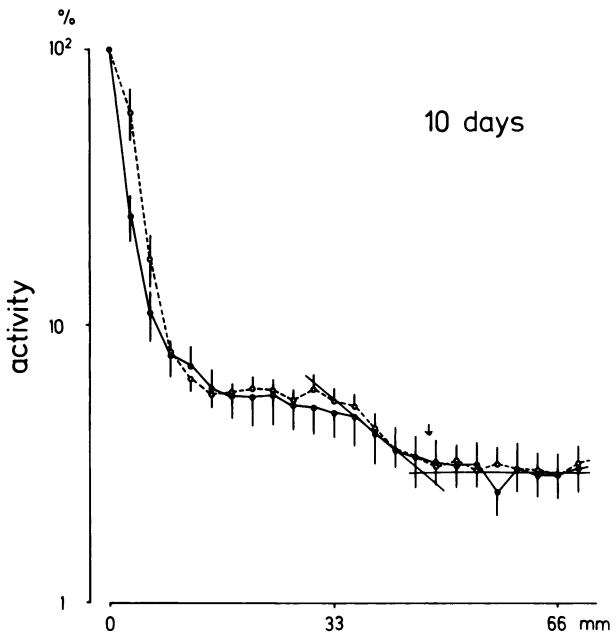

FIGURE 1 Distribution of protein-labeled activity in 3-mm segments along the sciatic nerve 10 d after the injection of tritiated proline into the fifth lumbar dorsal root ganglion. – – –, the mean curve for the group of diabetic rats; ——, curve for the controls. The activity in the ganglion is put equal to 100%, and the amount of activity in each 3-mm segment is expressed relative to this. Values are mean±SEM. The position of the wave front, indicated by the arrow, was the intercept between the line through the wavefront and the line through the background activity ahead of the front as shown for the control curve. In the experiment, the determination was made on individual animals.

The peak velocity was 0.93±0.047 and 0.76±0.099 mm/d for the control and the diabetic group. The individual values for the peak velocity are shown in Fig. 3. The slowing of the peak velocity is 19% in diabetics and the difference is statistically significant ($2p = 9.1 \times 10^{-5}$).

After 50 d a similar slowing of SCa in diabetics was obtained. The mean velocity was 0.76±0.051 and 0.64±0.049 mm/d for the control and the diabetic rats, respectively, and the 16% difference is statistically significant ($2p = 1.2 \times 10^{-5}$). The velocity of the peak was 0.77±0.078 mm/d in controls and 0.62±0.041 mm/d in diabetics. The values obtained for each animal are shown in Fig. 3. The difference again amounts to 19% ($2p = 2.7 \times 10^{-5}$).

Amount of transported material. The amount of material conveyed in SCa was expressed relative to the ganglion activity. After 25 d the amount for the control group was 76.0±0.28%, as opposed to 55.7±0.37% for the diabetic group. Thus, the diabetic axons conveyed 27% less labeled protein activity, but the difference is not statistically significant ($2p = 8.2 \times 10^{-2}$).

After 50 d the amounts were 72.3±0.45 and 54.6 ±0.55% in controls and diabetics, respectively. The reduction amounts to 24% but is statistically insignificant ($2p = 2.8 \times 10^{-1}$).

These results show that the amount in transport expressed relative to ganglion activity is unchanged in both groups from day 25 to day 50. The constancy of this fraction makes it likely that the turnover rate of labeled protein in the ganglion is similar to the turnover rate of labeled protein in axonal transport. There-

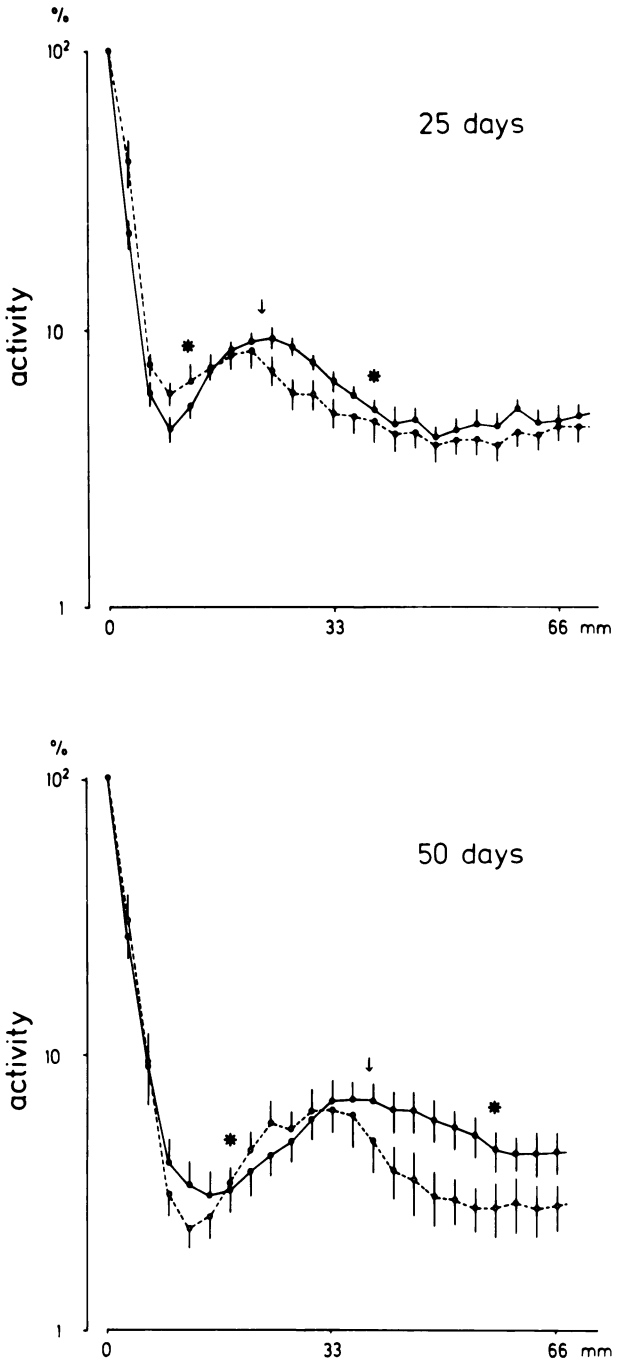

FIGURE 2 Distribution of protein-labeled activity in 3-mm segments along the sciatic nerve 25 and 50 d after the injection of tritiated proline into the fifth lumbar dorsal root ganglion. – – –, mean curves for the groups of diabetic rats; ——, curves for the controls. The ordinate is explained in Fig. 1. Values are mean±SEM. The first and the last segment under the bell-shaped curve are indicated by asterisks and the point of peak activity by the arrow. Note that the peak of activity has advanced for a shorter distance from the ganglion in diabetics than in controls at both intervals.

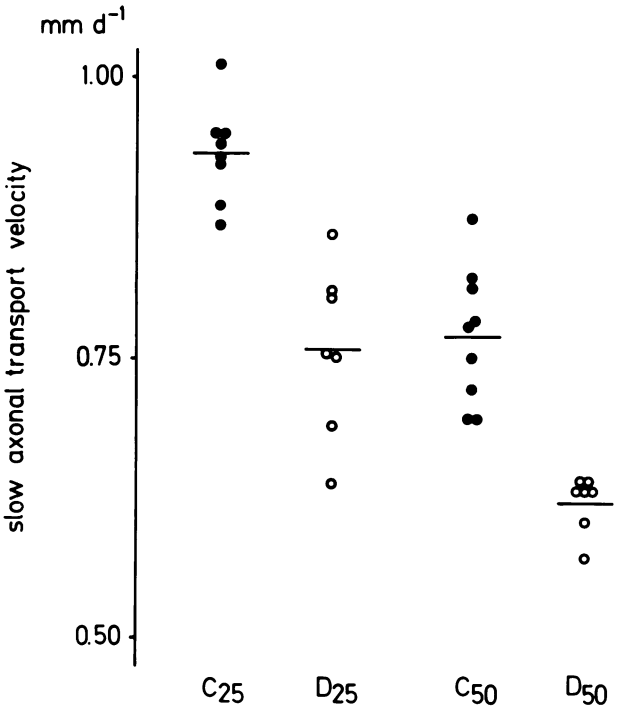

FIGURE 3 Individual values for the velocity of the peak of labeled proteins in SCa of the axonal transport system in control (●) and diabetic (○) rats obtained 25 and 50 d after the injection of tritiated proline into the fifth lumbar dorsal root ganglion. Note that there is no overlap of values between diabetic (D) and controls (C) after either 25 or 50 d.

fore, turnover of labeled ganglion protein is considered to estimate the turnover of proteins of SCa of the slow axonal transport.

Turnover of labeled ganglion protein. The protein turnover was estimated from the ganglion activity in each animal of all the experimental groups.

The regression line for the amount of activity in the ganglion (A_{ggl}) of the control group was $\ln A_{ggl} = 11.8 - 0.0318\ d$ ($n = 33$) as compared with $\ln A_{ggl} = 12.3 - 0.0395\ d$ ($n = 30$) for the diabetic group. Statistical analysis revealed no differences between the two lines. The slope of the regression line for the control group, -0.0318 ± 0.38, is equivalent to a half-life of 21.8 d. The slope for the diabetic group, -0.0395, corresponds to 17.5 d.

DISCUSSION

The present experiment has demonstrated that SCa of the axonal transport system is advanced more slowly in streptozotocin diabetic rats than in controls.

The transport velocity for SCb was normal in diabetic rats; the fast transport velocity was also normal. The analysis of the disappearance rate of the activity

incorporated in the ganglion and the fraction conveyed by SCa shows that the decreased velocity of this component is not compensated for by an increased protein synthesis, a prolonged half-life, or an increase in the relative amount of transported material.

A significant finding to basic knowledge on slow axonal transport is the present demonstration of decremental advancement of structural proteins in the rat. Slow axonal transport is known to be faster in younger animals (4, 12, 18–20). Because the rats of the 50-d group were injected 25 d earlier than the rats in the 25-d group, the difference in velocity cannot be explained by age. A consequence of the finding of decremental advancement is that estimation of slow transport velocity in pathologic and normal conditions must be compared at identical time intervals. A decremental advancement of SCa also appears in Table I of a report by Lasek (21), though the finding was not commented upon.

In a study of the polypeptides in the slow component of the axonal transport system in the rat ventral motor neuron and the cat spinal ganglion sensory neuron, Hoffman and Lasek (11) observed that only five polypeptides account for more than 75% of the proteins. Two of these polypeptides were tentatively identified as tubulin; the three remaining polypeptides were suggested to be constituents of neurofilaments. The suggestion is supported by the demonstration of a similar triplet as the major component of intact neurofilaments (22).

The interpretation of the decreased velocity in the diabetic group as well as of the decremental advancement of the wave of activity can be either a decrease in velocity of the individual proteins or a relative change in the amount of different proteins each moving at its own constant velocity. Future experiments shall clarify this question.

Our previous studies have shown that the axon calibre is 14–20% smaller in streptozotocin rats after a 4-wk duration of diabetes (1, 2). Morphometric studies of the axonal ultrastructure have demonstrated that the density of neurofilaments is unchanged in this model (1); recently we have found that the same is valid for microtubulus density (unpublished observation from material of a previous study [1]). Because the densities are unchanged in the diabetic rats and the axon calibre is smaller, the total amount of microtubules and neurofilaments is reduced. The decreased amount of the main structures of the axon could be obtained either by an increased turnover of structural proteins or by a decreased transport of structural proteins. The present experiments have not given any evidence for a changed turnover of structural proteins in streptozotocin diabetes.

The half-life of labeled proteins in the ganglion was unchanged and the relative amount of material transported in SCa remained nearly constant after 25 and 50 d in diabetics as well as in controls. It might be speculated whether the decreased transport of proteins could be secondary to the changes in axon calibre. However, the axon calibre of diabetic rats corresponds to that of younger animals in whom the transport is faster. Consequently, the finding of a decreased transport velocity of SCa in streptozotocin diabetes in the rat is concluded to be the likely explanation for the smaller axon calibre.

We have demonstrated earlier that insulin treatment can prevent the reduction in axon calibre (1), indicating that the changes are not the result of a toxic effect of streptozotocin itself. We have also shown that the axon calibre remains normal in rats fed a restricted diet, leading to a weight loss comparable with that of the diabetic animals (2). Therefore, it seems likely that the decrease of the slow axonal transport velocity is the result of the metabolic derangement rather than of the weight loss occurring in the diabetic animals.

How does diabetes influence the transport velocity of SCa? We have recently reported that the amount of retrogradely transported glycoproteins within the axons is decreased in streptozotocin diabetic rats (14, 15). Materials carried by retrograde axonal transport possibly influence the protein synthesis in the cell body (23–25), and we have indeed demonstrated structural changes of the nerve cell bodies in this model (26). Therefore, we suggest that the impaired retrograde transport is the trigger for changes in synthesis of protein in the nerve cell body and in turn is responsible for the decreased slow axonal transport.

The relevance of these studies for human diabetic neuropathy shall finally be mentioned. Primary axonal degeneration is known to occur in human diabetics even at the time of diagnosis of the metabolic disturbance (27). A decrease in the transport of structural proteins in diabetic patients might well be an explanation of these structural axon changes.

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