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Distal axonopathy in streptozotocin diabetes in rats

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Summary. We noted the earliest morphological changes in the motor endplates 8 weeks after the induction of streptozotocin diabetes in rats. Morphometric measurements showed reduced axonal areas of the lateral plantar and the sciatic nerves in the diabetic rats 28 but not 2 and 8 weeks after the experiment. These findings suggested distal axonopathy.
Key words. Streptozotocin; rats; diabetic; distal; axonopathy.

The pathogenesis of diabetic neuropathies remains elusive[1,2] and the search for underlying mechanisms is hampered by the lack of a satisfactory animal model. Nerve conduction studies have shown reduced conduction velocities early in the course of experimental diabetes[3–5], however, morphological correlates have not been consistently identified. Several morphological abnormalities in the somatic nerves have been reported: axonal degeneration and atrophy[6], endoneurial edema[6], distal axonopathy[7,8], demyelinating neuropathy and axonal degeneration[9], axonal shrinkage[10] due to hyperosmolar state rather than axonal atrophy and a lack of structural alterations, and minor changes related to growth inhibition and maturational deficits in diabetic animals[11]. We wish here to report morphological changes in the distalmost part of the lower motor neuron of streptozotocin-induced diabetic rats proceeding proximally in the course of time.

Methods. Charles River Strain (Charles River Breeding Company, Wilmington, Mass.) of Sprague-Dawley adult male rats weighing 180–200 g were injected with streptozotocin (50 mg/kg b. wt) into the tail vein to induce experimental diabetes mellitus. All rats were housed in cages under identical circumstances and given free access to water and Purina Rat Chow. We followed the Institution's guide for the care and use of laboratory animals. Age and weight matched male rats were used for controls receiving no injections. There were 5–9 rats in each group (diabetic vs control).

Before sacrifice at the end of 2, 8 and 28 weeks, blood was collected from the tail vein of each animal for glucose assay. The animals were then weighed and lightly anesthetized with nembutal. Perfusion-fixation of the hind limbs was accomplished at room temperature through the abdominal aorta with 2.5% glutaraldehyde in 0.2 N cacodylate buffer at a pH of 7.2. A strip of muscle was removed from the medial gastrocnemius muscle in the region of the motor points and fixed in 2.5% glutaraldehyde for studying motor endplate fine structure. Portions of the sciatic nerve from the proximal and distal segments, the tibial nerve close to its insertion into the medial gastrocnemius muscle and the lateral plantar nerve were removed and fixed in glutaraldehyde.

We used a modification[12] of Engel's technique for localizing the endplate for electron microscopy. A Zeiss MOP-3 manual optical picture analyzer was used to measure the presynaptic and postsynaptic membrane length, the nerve terminal and postsynaptic areas of the motor endplate fine structure. The nerve specimens were embedded in an epon-araldite mixture, and semithin sections (1–1.5 μm in thickness) in cross sections were stained with toluidine blue and photographed at a final magnification of 1000 x for measurement of the axonal areas. Ultrathin sections of the nerves were then cut on an ultramicrotome and examined by electron microscopy.

Morphological data were subjected to qualitative and quantitative analysis using a 2-tailed Student's t-test. The dependent variables were also analyzed using analysis of variance (ANOVA).

Results. Rats injected with streptozotocin remained diabetic as evidenced by hyperglycemia and glucosuria throughout the experiment. They lost weight considerably in the first 2 weeks, but then showed a slight weight gain which was much less than the weight gained by the control rats (table 1). A two-way (group × time) ANOVA was performed with time as a repeated measure on the variables, weight change and blood glucose (table 1). No significant relationship was found between the blood glucose and weight loss between the diabetic and control animals. The diabetic rats developed cataracts after 8 weeks.

Morphological observations. There was no significant difference between the values in different segments of the sciatic-tibial-lateral plantar nerves in the control and diabetic rats at

Table 1. Mean weight (g) change (gain +; loss −) and mean blood glucose (mg/dl) in diabetic and control rats

Group	Determination	2 weeks	8 weeks	28 weeks
Control rats	Blood glucose	89.0	86.3	91.1
	Weight change	+ 72.0	+ 181.3	+ 335.6
Diabetic rats	Blood glucose	320.3	338.7	424.2
	Weight change	− 72.7	+ 1.9	+ 22.2

Table 2. Mean ± SEM of the axonal area (μm^2) at 4 levles of the peripheral nerves in diabetic (D) and control (C) rats 2, 8 and 28 weeks after the onset of the experiment

Determination group		Proximal sciatic nerve (myelinated) 2	8	28	Distal sciatic nerve (myelinated) 2	8	28	Tibial nerve (myelinated) 2	8	28	Lateral plantar nerve (myelinated) 2	8	28	Lateral plantar nerve (unmyelinated) 2	8	28
Axonal area	D	10.4 ± 0.8 n = 8	14.5 ± 0.8 n = 8	16.5 ± 2.2 n = 5	*7.3 ± 0.7 n = 8	13.6 ± 0.8 n = 8	*12.0 ± 1.3 n = 5	7.8 ± 1.3 n = 8	12.3 ± 1.3 n = 8	11.8 ± 1.3 n = 5	7.2 ± 0.3 n = 8	8.9 ± 0.9 n = 8	*7.1 ± 0.4 n = 5	–	–	*0.4 ± 0.04 n = 5
Axonal area	C	11.0 ± 2.0 n = 6	16.0 ± 1.3 n = 7	20.3 ± 0.4 n = 5	12.7 ± 1.0 n = 8	15.5 ± 2.2 n = 8	17.7 ± 1.8 n = 5	7.6 ± 0.8 n = 8	14.7 ± 1.6 n = 8	15.6 ± 2.3 n = 5	8.1 ± 0.6 n = 8	8.8 ± 0.6 n = 8	10.7 ± 0.4 n = 5	–	–	0.7 ± 0.15 n = 5

n = number of rats; – = not measured, * = significantly smaller ($p < 0.05$).

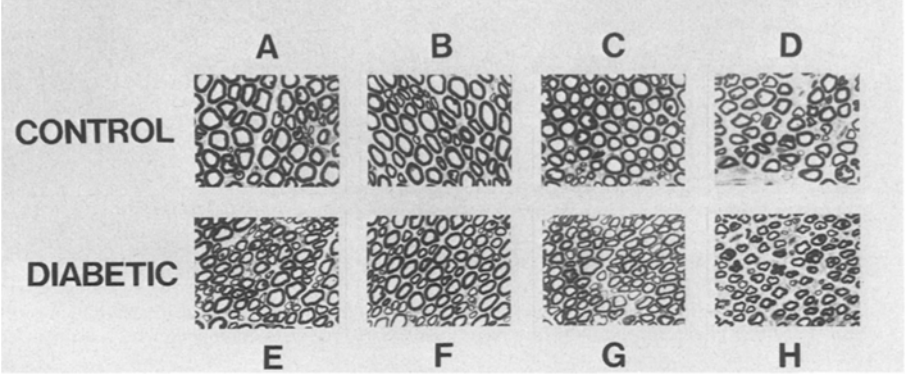

Figure 1. Semithin transverse sections of portions of peripheral nerves at 4 levels in control (A: proximal sciatic; B: distal sciatic; C: tibial branch to medial gastrocnemius muscle; D: lateral plantar) and diabetic (E: proximal sciatic; F: distal sciatic; G: tibial; H: lateral plantar) rats 28 weeks after the onset of the experiment.

the end of 2 and 8 weeks (table 2) of the experiment ($p > 0.05$) except for a small axonal area in the distal sciatic nerve of the diabetic rats after two weeks (table 2); this latter finding may be spurious. However, 28 weeks after the experiment the mean axonal areas of the myelinated fibers in the lateral plantar and the distal sciatic nerves (fig. 1; table 2), and the mean myelinated axonal area obtained by summating the mean values of the proximal and distal sciatic, tibial and lateral plantar nerves assumed statistical significance ($p < 0.05$), and were smaller in the diabetic than in the control rats. The mean axonal areas of the unmyelinated fibers were significantly smaller ($p < 0.05$) in the diabetic than in the control rats 28 weeks but not 2 and 8 weeks after the onset of the experiment (table 2). Only minor degenerative changes without evidence of demyelination or remyelination in the myelinated axons were seen in the diabetic rats 28 weeks after the onset of the experiment.

Many endplates in the diabetic rats after 8 weeks showed evidence of degeneration, e.g., myeloid bodies, stacks of membranous saccules in the nerve terminal regions and postsynaptic regions denuded of nerve terminals in the plane of section (fig. 2). These conformational changes were more frequent in the diabetic than in the control rats ($p < 0.05$) using a Chi-square test. We measured the presynaptic and postsynaptic profiles in 57 motor endplates containing 194 nerve terminals in 6 diabetic and 6 control rats 2 weeks after the onset of the experiment; 65 motor endplates containing 189 nerve terminals in 8 diabetic and 6 control rats 8 weeks after the onset of the experiment; and 76 endplates containing 353 nerve terminals in 5 diabetic and 5 control rats 28 weeks after the onset of the experiment. Eight weeks after injection there was enlargement ($p < 0.05$) of the mean nerve terminal area in the diabetic rats and the number of small nerve terminals was reduced. The presynaptic and postsynaptic profiles did not differ between the control and diabetic rats 2 and 28 weeks after the onset of the experiment.

Discussion. Definite evidence of abnormalities in the fine structure of the motor endplates (e.g., enlargement of the nerve terminal area and a decrease in the number of small nerve terminals) of diabetic rats was found in the eighth week after the onset of diabetes mellitus. These fine structural changes are reminiscent of those described in motor endplates in acrylamide neuropathy by Tsujihata et al.[13] and appear to be consistent with distal axonal degeneration.

We failed to find any changes at 4 levels in the somatic nerves in the proximal and distal regions in the diabetic rats 2 and 8 weeks after induction of streptozotocin-induced diabetes mellitus. Our observations on the somatic peripheral nerves in early experimental diabetes (up to 8 weeks of experiment) conflict with some previous reports in the literature[6,7,9] but generally agree with those of Sharma and Thomas[4,11]. Our data do not support growth retardation as a factor for axonal atrophy. We did not find any significant axonal atrophy in the diabetic rats at the end of 2 and 8 weeks of experiment when the maximum failure to gain weight was noted. Furthermore, axonal atrophy related to retardation of growth should be reflected uniformly in all segments and not necessarily in the distal segments only.

Our morphometric data showing enlargement of the nerve terminal area of the medial gastrocnemius motor endplates, the presence of stacks of membranous saccules and myeloid bodies in the nerve terminals and the presence of excessive numbers of postsynaptic regions denuded of nerve terminals in the plane of section in the diabetic rats 8 weeks after

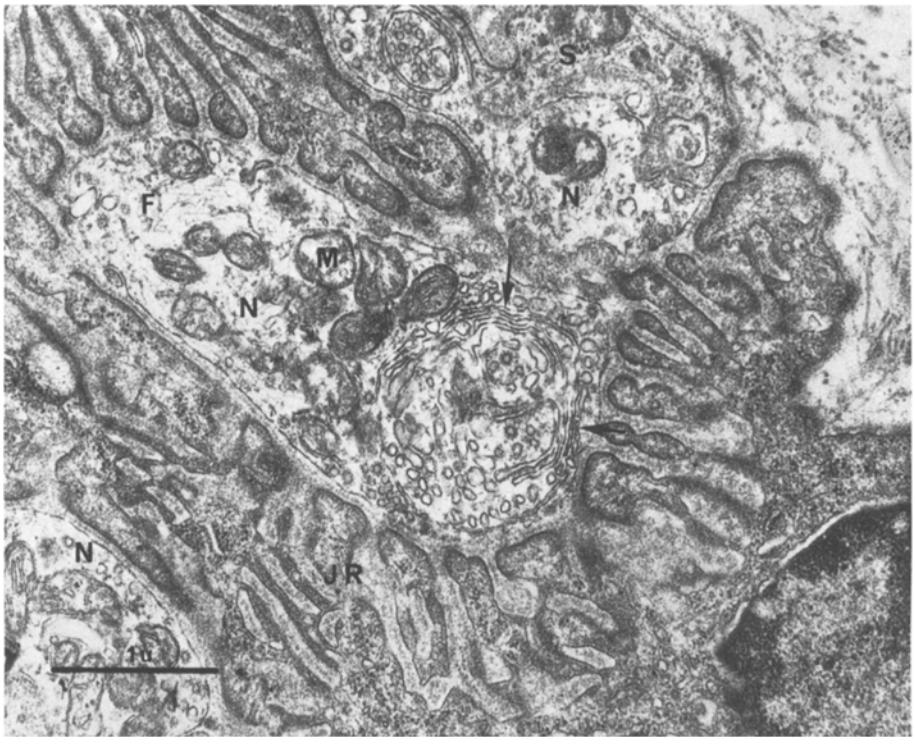

Figure 2. Portion of a motor endplate of the medial gastrocnemius muscles of a diabetic rat 8 weeks after onset of experiment. Note one nerve terminal (N) and portions of two other nerve terminals. Arrow points to stacks of membranous saccules. F, neurofilaments; M, mitochondria; JR, junctional region of folds and clefts; scale bar: 1 μm.

streptozotocin injection indicate that the earliest changes may begin in the motor endplates 8 weeks but not 2 weeks after the induction of experimental diabetes mellitus. Such morphological changes first in the gastrocnemius motor endplates and then proceeding proximally to affect the tibial and sciatic nerves in the course of time suggest a spatio-temporal pattern of evolution of these abnormalitites. Furthermore, significant reduction in the axonal areas of the lateral plantar nerve in the diabetic rats 28 weeks but not 2 and 8 weeks after onset of the experiment support such evolution. The previous studies on streptozotocin diabetes generally did not show such distal to proximal progression in the course of time. These findings are the hallmark of distal axonopathy and a streptozotocin rat model satisfies the criteria for diabetic distal axonopathy.

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