NT-3 Attenuates Functional and Structural Disorders in Sensory Nerves of Galactose-fed Rats

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Abstract. The present study investigated the effect of NT-3, a neurotrophin expressed in nerve and skeletal muscle, on myelinated fiber disorders of galactose-fed rats. Adult, female Sprague-Dawley rats were fed diets containing complete micronutrient supplements and either 0% D-galactose (control) or 40% D-galactose. Treated controls received 20 mg/kg NT-3 and treated galactose-fed rats received 1, 5, or 20 mg/kg NT-3 three times per week by subcutaneous injections. After 2 months, sciatic and saphenous sensory nerve conduction velocity (SNCV) and sciatic motor nerve conduction velocity (MNCV) were measured and the sciatic, sural, peroneal and saphenous nerves and dorsal and ventral roots processed for light microscopy. Treatment of control animals with NT-3 had no effect on any functional or structural parameter. Compared to control values, galactose feeding induced a sensory and motor nerve conduction deficit and a reduction in axonal caliber. Treatment with 5 and 20 mg/kg NT-3 ameliorated deficits in sciatic and saphenous SNCV in galactose-fed rats but had no effect on the MNCV deficit. NT-3 treatment also attenuated the decrease in mean axonal caliber in the dorsal root and sural nerve but not in the saphenous nerve, ventral root and peroneal nerve. These observations show that NT-3 can selectively attenuate the sensory conduction deficit of galactose neuropathy in a dose-dependent manner that depends only in part on restoration of axonal caliber of large-fiber sensory neurons.

Key Words: Aldose reductase; Galactose intoxication; Neurotrophin-3; Peripheral nerve; Polypeptide pathway; Skeletal muscle.

INTRODUCTION

Hyperglycemia-induced flux through the polypeptide pathway has been associated with many complications resulting from experimental or clinical diabetes. The key anabolic enzyme of the pathway, aldose reductase (AR), reduces a variety of hexoses, including glucose and galactose, to their respective polyols, sorbitol and dulcitol. The ability of aldose reductase inhibitors (ARIs) to prevent biochemical, functional and structural abnormalities has implicated flux through the pathway and/or polypeptide accumulation in the etiology of many diabetic complications, including neuropathy, retinopathy, and nephropathy (1, 2). In peripheral nerve, ARIs prevent or ameliorate the conduction velocity deficits and axonal dwindling characteristic of experimental diabetic neuropathy (3). However, in spite of the widely documented efficacy of ARIs in preventing nerve dysfunction, it is not clear how inhibition of an enzyme localized to Schwann cells and skeletal muscle (4–9) prevents nerve conduction abnormalities (10, 11) and restores axonal caliber (12, 13).

One explanation for why nerve dysfunction in experimental diabetes is ARI-sensitive may be that flux through the polypeptide pathway or polypeptide accumulation in Schwann cells and skeletal muscle disrupts the synthesis, release and/or transport of neurotrophic factors required for the maintenance of axonal function and structure. Evidence supporting this notion derives from recent studies demonstrating altered levels of neurotrophic factors in rodent models of experimental diabetes and from studies showing neurotrophin-induced protection from neurofilament loss after nerve injury. In this regard, Schwann cell deficits in ciliary neurotrophic factor (CNTF) bioactivity and protein levels in experimental diabetes are partially restored by AR inhibition (14, 15). Although associations between other neurotrophic factors and flux through aldose reductase have not been reported, levels of NGF, BDNF and NT-3 mRNA and/or protein are altered in nerve and muscle of diabetic or galactose-fed rats (16–20). A link between neurotrophic support and neurofilaments, the cytoskeletal component that determines axonal caliber and thus influences nerve conduction velocity, is supported by studies demonstrating recovery of neurofilament loss in injured neurons following exogenous administration of the target-derived neurotrophins, NGF or BDNF (21, 22).

Substantial polypeptide accumulation occurs after as little as 1 week of galactose feeding, because dulcitol is not further oxidized (23, 24). This, coupled with normal insulin levels in galactose-fed animals, has established galactose intoxication as a model of polypeptide pathway complications free from the consequences of insulin deficiency. Because we have previously shown that BDNF ameliorates motor, but not sensory conduction velocity deficits in galactose-fed rats (20), it is of interest to study NT-3, which may affect different neuronal populations than BDNF (25). In a preliminary report (26), NT-3 attenuated the sensory conduction deficit in the sciatic nerves of galactose-intoxicated rats, but had no effect on motor nerve conduction velocity.
(MNCV). The present study was designed to determine the dose-dependence of this effect, confirm it in a pure sensory nerve, and explore possible structural correlates. We now report that NT-3 selectively attenuates functional and structural deficits of large fibers in sensory nerves of galactose-fed rats in a dose-dependent fashion.

METHODS

These studies were conducted with the approval of the San Diego Veterans Administration Animal Studies Subcommittee. Over 2 months, age-matched, female Sprague-Dawley rats (200–250 g; Charles River, San Diego, Calif.) were weighed weekly until body weight was stable at which time onset sciatric motor nerve conduction measurements were made. Groups were then established and rats fed either a 40% D-galactose diet or a control diet specially formulated by Purina (Richmond, IN) that contained 40% solka floc, a nonnutritive fiber, to balance nutritional intake. All diets contained 100% of the micro-nutrients required by rats, and with water, were available ad libitum. Each of these diet groups contained animals treated with human recombinant NT-3, given by subcutaneous injection three times per week. Control animals were treated with vehicle (10 mM histidine, 0.5% sucrose, 4.5% mannitol, pH 5) only or 20 mg/kg NT-3, while galactose-fed animals received either vehicle only or 1, 5 or 20 mg/kg NT-3. The NT-3 doses were based on a previous study (27).

Prior to beginning diets and NT-3 treatment, rats were anesthetized with halothane (5% for induction and 2% for maintenance of anesthesia) before measurement of onset sciatric MNCV (see below). After 2 months of diet and treatment, rats were anesthetized by intraperitoneal injection (2 ml/kg) of pentobarbital sodium (12.5 mg/ml) and diazepam (1.25 mg/ml) in bacteriostatic physiological saline. Under deep anesthesia, conduction velocity measurements were made in the hindlimb. Subsequently, in some animals, samples of sensory and motor components of the peripheral neuraxis were removed after perfusion fixation and processed for light microscopic examination (see below).

Sciatic and saphenous nerve conduction velocities were measured with nerve temperatures maintained at 37°C. For the sciatic measurements, the right sciatic nerve was minimally exposed at the sciatic notch and a bipolar needle stimulating electrode was placed around the nerve trunk at the level of the obturator tendon. A second bipolar stimulating electrode was inserted percutaneously at the ankle with the reference electrode inserted into the base of the tail. Supramaximal square wave stimulations (50 μs pulse width; 58019 Square Wave Stimulator, Stoelting Co., Chicago, Ill.) were applied to the nerve and the resulting muscle compound action potentials recorded from the interosseus muscles of the ipsilateral foot with 2 needle electrodes. Recordings were first made for sensory H waves, monosynaptically evoked reflexes resulting from stimulation (2–5 V) of afferent proprioceptive Ia fibers which excite spinal cord α-motor neurons (28, 29). The stimulation voltage was increased 5–10 V for recordings of the motor M waves. All signals were amplified with a P15 AC Amplifier (Grass Instruments Co., Quincy, Mass.) and displayed on a 5110 Storage Oscilloscope with 5D10 Waveform Digitizer (Tektronix Inc., Beaverton, Oreg.). The median latency difference between three pairs of notch- and ankle-evoked H or M wave responses was recorded for each nerve in a fully extended hindlimb. The distance between stimulation sites with the hindlimb extended was divided by the median latency difference between H wave responses to calculate sciatric SNCV. The latency difference between M wave responses was similarly used to calculate sciatric MNCV.

Saphenous SNCV was recorded from the contralateral limb after the sciatic nerve measurements were made. The saphenous nerve was exposed between the groin and ankle and covered with a film of petroleum jelly to prevent desiccation. A bipolar needle stimulating electrode was placed around the nerve trunk just distal to the inguinal ligament, recording hook electrodes were placed around the nerve trunk in the lower leg, and a reference electrode was inserted into the base of the tail. Stimulation was just supra-maximal (2–5 V, 50 μs pulse width). The distance between stimulation and recording sites with the hindlimb extended was divided by the median latency difference between the beginning of the stimulus artifact and the first inflexion of 3 responses to give the conduction velocity of the fastest conducting sensory fibers.

Sensory (sural and saphenous nerves and L5 dorsal roots) and motor (peroneal nerves and L5 ventral roots) populations of myelinated fibers were obtained from animals perfused through the heart with 200 ml of heparinized, phosphate-buffered saline (37°C) followed by 250 ml of 2.5% phosphate-buffered glutaraldehyde. The nerves and roots were then removed and fixed overnight in 2.5% phosphate-buffered glutaraldehyde (4°C). Tissue was postfixed in 1% phosphate-buffered osmium tetroxide for 3–4 hours (h) before dehydration using a series of graded alcohols and propylene oxide. After infiltration with a 1:1 mixture of propylene oxide and araldite for 4 hours, nerves and roots were placed in 100% araldite overnight before embedding in fresh araldite resin. Thick sections (1 μm) were cut with glass knives and stained with p-phenylenediamine prior to light microscopic examination.

Computer-assisted analyses of axonal size-frequency distributions of myelinated fibers were performed on nerves and roots. For each of the nerves, a single thick section was analyzed as described previously (30). For the sural and peroneal nerves, thick sections came from tissue sampled at the knee just distal to the trifurcation of the sciatic nerve. The saphenous nerve was sampled midway between the inguinal ligament and knee. For the L5 dorsal and ventral roots, single sections from each animal were sampled midway between the dorsal root ganglion and the root entry zone (20). For each nerve or root, a video image was obtained with an Olympus BH-2 light microscope and Cohu 5000 series television camera interfaced with a Macintosh Quadra 850AV computer running NIH Image 1.55 software. Myelinated fibers with axons greater than 1 μm in diameter were individually identified and selected prior to being sorted with an automated process into bins based on axonal diameter. Prior to and during analysis, video images of myelinated fibers were checked against the light microscopic images in order to assess axonal integrity and insure that only internodal profiles were sampled. Axons of myelinated fibers with profiles including the Schwann cell nucleus or paranode were not analyzed. In each case, the whole fascicle was systematically sampled with nonoverlapping fields, resulting in sampling of 50–80% of the total myelinated fibers at each site.
TABLE 1
Onset and Final Body Weight and Motor Nerve Conduction Velocity (MNCV)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body weight (g)</th>
<th>MNCV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All animals</td>
<td>60</td>
<td>304 ± 19^</td>
<td>54.6 ± 4.9</td>
</tr>
<tr>
<td><strong>Final</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>300 ± 22</td>
<td>52.3 ± 5.6</td>
</tr>
<tr>
<td>Control + NT-3</td>
<td>10</td>
<td>303 ± 15</td>
<td>51.8 ± 5.7</td>
</tr>
<tr>
<td>(20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>10</td>
<td>276 ± 11^</td>
<td>46.8 ± 3.5</td>
</tr>
<tr>
<td>Galactose + NT-3</td>
<td>9</td>
<td>279 ± 14^</td>
<td>46.1 ± 6.6</td>
</tr>
<tr>
<td>(1 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose + NT-3</td>
<td>10</td>
<td>276 ± 17^</td>
<td>46.5 ± 3.9</td>
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<tr>
<td>(5 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose + NT-3</td>
<td>9</td>
<td>277 ± 11^</td>
<td>46.8 ± 3.1</td>
</tr>
</tbody>
</table>

Prior to the beginning of the study, body weights and MNCV measurements were made on all animals. Final measurements were made after animals were maintained on 0% (control) or 40% galactose diets for 2 months during which time treated animals received subcutaneous injections of NT-3 3 times per week. Data are presented as mean ± SD and were analyzed with a one way ANOVA after which comparisons were made with Dunnett’s test. N = 8 for the final MNCV of the Galactose + NT-3.

Myelin splitting and ballooning in the dorsal and ventral roots were quantified using a Jenaval 250-CF light microscope and camera lucida attachment (20). In each root of the control, untreated galactose, and galactose + 20 mg/kg NT-3 groups, the number of fibers with evidence of myelin splitting or ballooning was counted. Fibers with splitting or ballooning were defined as those with clear, faintly staining space evident between separated myelin with an asymmetrical profile. Paranodal profiles or profiles with Schmidt-Lanterman clefts were not included in counts of myelin splitting.

All experiments and data collection were conducted with animals and tissues coded to eliminate bias. Differences occurring between groups were tested by one way ANOVA. If statistically significant (p < 0.05), multiple comparisons to the untreated control group were made with the Dunnett test, or between groups with the Student-Newman-Keuls test. When standard deviations were significantly different, groups were compared with the Kruskal-Wallis test followed by the method of Dunn. Data are presented as mean ± SD or median and range.

RESULTS

To verify that the rats in this study were mature, onset and final body weights and MNCV measurements were made (Table 1). After 2 months, body weight and MNCV of the untreated control rats were comparable to the onset body weight and MNCV of all animals. The final body weight and MNCV of those animals fed the galactose diet were significantly less than onset body weight and MNCV. NT-3 treatment did not appear to affect the final body weight or MNCV of either control or galactose-fed animals.

Because of our previous finding of selective effects on sciatic motor and sensory conduction velocity (26), the dose-dependent effects of NT-3 on nerve conduction velocity were examined after 2 months of galactose feeding (Tables 1, 2). Measurements of saphenous nerve conduction velocity were included to verify effects in a pure sensory nerve. The SNCV and MNCV of untreated galactose-fed animals were significantly less than those of untreated controls. Although MNCV of the untreated galactose group just missed statistical significance when compared to age-matched controls (Table 2), the difference was statistically significant when compared to onset control MNCV (Table 1). NT-3 treatment of control animals was without effect on SNCV of the sciatic or saphenous nerves. However, despite having no effect on body weight, NT-3 treatment ameliorated the SNCV deficit in both the sciatic and saphenous nerves of galactose-fed animals in a dose-dependent fashion. In contrast, NT-3 treatment had no impact on the galactose-induced MNCV deficit.

As a first attempt to examine the structural correlates of NT-3 treatment, mean axonal diameters of sensory and motor components of the peripheral neuraxis were assessed (Table 3). In the dorsal and ventral roots, sural, peroneal, and saphenous nerves, galactose feeding was associated with a significant decrease in mean axonal diameter of...
myelinated fibers compared to control animals. The decrease in axonal caliber was not associated with loss of myelinated fibers at any site other than the ventral root (data not shown). In the dorsal root and sural nerve of NT-3-treated, galactose-fed animals, this decrease was attenuated such that the mean axonal diameter was significantly greater than that of untreated galactose-fed rats, but significantly less than that of controls. NT-3 treatment had no effect on the mean axonal diameter of the saphenous and peroneal nerves or ventral roots of galactose-fed rats.

To further examine morphological correlates, the axonal size-frequency distributions of myelinated fibers from sensory and motor populations were determined (Figs. 1, 2). In the dorsal root and sural nerve, there was an apparent shift in the axonal size-frequency distribution of untreated, galactose-fed rats towards smaller diameters that appeared to be attenuated by NT-3 treatment (Fig. 1A, C). Analysis of the relative number of myelinated fibers with axonal diameters greater than or equal to 6 μm demonstrated that NT-3 treatment of galactose-fed animals significantly attenuated the decrease in the number of large myelinated fibers in the dorsal root (Fig. 1B) and sural nerve (Fig. 1D). In contrast, neither a shift in the axonal size-frequency distribution of myelinated fibers (Fig. 2C) nor the decrease in the relative number of large fibers (Fig. 2D) in the peroneal nerve of galactose-fed rats were affected by NT-3 treatment. In the ventral root, the decreased percentage of larger myelinated fibers (Fig. 2A) was obscured by using an axonal diameter cutoff of greater than or equal to 6 μm (Fig. 2B).

To assess the dependence of nerve conduction velocity on axonal caliber, the relationship between the mean values of these parameters was plotted (Fig. 3). In the dorsal root and sural nerve, dose-dependent increases in mean axonal diameter are associated with dose-dependent increases in SNVC (Fig. 3A, B). In contrast, neither mean axonal diameter nor MNCV in the ventral root or peroneal nerve change in response to NT-3 treatment (Fig. 3C, D).

As seen earlier (20), myelin splitting was prominent in dorsal and ventral roots of galactose-fed, but not control, rats (Fig. 4). The prevalence of myelin splitting in the galactose group appeared to be less in the ventral root (Table 4). Note that 1 galactose-fed animal had only 7 fibers with evidence of myelin splitting. In both dorsal and ventral roots of the galactose-fed rats, there was no significant effect of NT-3 treatment on myelin splitting.

**DISCUSSION**

The restoration of nerve conduction by inhibiting AR may reflect the effect of polyl pathway activity on the expression of neurotrophic factors in glial cells or other target tissues able to influence axonal function and structure. Altered mRNA and/or protein expression of CNTF, NGF, BDNF and NT-3 in nerve, muscle and other target tissues of diabetic and galactose-fed rats (15–20, 31) is consonant with hyperglycemia-induced polyl pathway activity affecting the expression of neurotrophic factors. These data have prompted the use of exogenously administered NGF, BDNF and NT-3 to correct altered neurotropic expression (32) or functional and structural disorders of experimental diabetic neuropathy (20, 31). The results of the present study extend the efficacy of NT-3 from streptozotocin diabetes to galactose intoxication and show that subcutaneous administration of this neurotrophin ameliorates both conduction deficits and axonal dwindling of sensory nerves in a dose-dependent fashion (Tables 2, 3; Figs. 1, 3). Together, these observations implicate altered neurotrophic support in the development of experimental diabetic neuropathy and provide an experimental rationale for considering the use of neurotrophins in clinical trials for the treatment of this disorder.

NT-3 is present in the nervous system and in a variety of nonneural tissues including skin, heart and skeletal...
Fig. 1. Axonal size-frequency distribution of myelinated fibers in the dorsal root and sural nerve of control and galactose-fed rats. Animals were maintained on 0% (control) or 40% galactose diets for 2 months during which time treated animals received subcutaneous injections of NT-3 3 times per week. A. Mean distribution in the dorsal root of control (11,785 fibers total), galactose (13,477 fibers total) and galactose + 20 mg/kg NT-3 (16,069 fibers total) groups. B. Relative number of myelinated fibers in the dorsal root with axonal diameters ≥ 6 microns. C. Mean distribution in the sural nerve of control (2,635 fibers total), galactose (3,242 fibers total) and galactose + 20 mg/kg NT-3 (3,334 fibers total) groups. D. Relative number of myelinated fibers in the sural nerve with axonal diameters ≥ 6 microns. Data are presented as mean ± SD (N = 5–7) and were analyzed with a one way ANOVA after which multiple comparisons were made with the Student-Newman-Keuls test.

Muscle (33). Mice carrying a deletion in either the gene for NT-3 or for the trkC receptor lack la proprioceptive afferents and muscle spindles (34, 35). In the adult, intrafusal fibers express NT-3 mRNA (36) and thus presumably provide neurotrophic support to the large proprioceptive fibers that express trkC and innervate muscle spindles. Decreased NT-3 expression in skeletal muscle of diabetic animals (18, 19, 31) and consequent effects
Fig. 2. Axonal size-frequency distribution of myelinated fibers in the ventral root and peroneal nerve of control and galactose-fed rats. Animals were maintained on 0% (control) or 40% galactose diets for 2 months during which time treated animals received subcutaneous injections of NT-3 3 times per week. A. Mean distribution in the ventral root of control (3,770 fibers total), galactose (4,228 fibers total) and galactose + 20 mg/kg NT-3 (5,318 fibers total) groups. B. Relative number of myelinated fibers in the ventral root with axonal diameters ≥ 6 microns. C. Mean distribution in the peroneal nerve of control (3,993 fibers total), galactose (4,864 fibers total) and galactose + 20 mg/kg NT-3 (6,933 fibers total) groups. D. Relative number of myelinated fibers in the peroneal nerve with axonal diameters ≥ 6 microns. Data are presented as mean ± SD (N = 4–7) and were analyzed with a one way ANOVA after which multiple comparisons were made with the Student-Newman-Keuls test.

on proprioceptive neurons may be a basis for the sensory conduction velocity deficits in experimental diabetic neuropathy. The restriction of treatment effects to sensory nerves of adult rats reported here (Tables 2, 3; Figs. 1–3) and elsewhere (31) is consistent with a restoration of trophic support to large proprioceptive Ia neurons by exogenous NT-3.

NT-3 treatment does not appear to prevent the MNCV deficit in adult rats with experimental diabetes (Tables 1, 2; 31). However, NT-3 promotes the survival of embry-
Fig. 3. Conduction velocity and mean axonal diameter in sensory and motor nerve fibers of control and galactose-fed rats. Sciatic SNCV versus mean axonal diameter of myelinated fibers in the dorsal root (A) and sural nerve (B). Sciatic MNCV versus mean axonal diameter of myelinated fibers in the ventral root (C) and peroneal nerve (D). Data are expressed as the mean ± SD (horizontal bar for mean axonal diameter; vertical bar for SNCV or MNCV) of control, control + 20 mg/kg NT-3, galactose, galactose + 1 mg/kg NT-3, galactose + 5 mg/kg NT-3 and galactose + 20 mg/kg NT-3 groups. Open and filled circles represent control and galactose-fed groups, respectively. Circle diameter indicates NT-3 dose: smallest and largest diameter circles denote untreated animals and those receiving the 20 mg/kg dose, respectively; intermediate sized diameters show 1 and 5 mg/kg doses. Mean values are taken from data in Tables 2, 3.

Onic motor neurons in vitro (37) and neonatal motor neurons in vivo (38). Moreover, these neurons express trkC receptors and transport NT-3 in a retrograde fashion to the ventral horn (25, 38). Therefore, it is not clear why motor neurons do not respond to exogenous NT-3 treatment in experimental diabetes unless the factors mediating the ability to respond to NT-3 are restricted to sensory neurons later in postnatal development.
Fig. 4. Sensory and motor components of the peripheral neuraxis in control and galactose-fed rats. Dorsal roots from control (A) and galactose-fed (B) rats. Note that the fibers in the control have compact myelin sheaths while many fibers in the dorsal root from the galactose-fed rat show evidence of myelin splitting and ballooning. Ventral roots from control (C) and galactose-fed (D) rats. Myelin with symmetric and circumferential widening of lamellae likely represent Schmidt-Lantermann incisures. As in the dorsal root, myelin splitting and ballooning are prominent in the ventral root of galactose-fed rats. Sural nerves from control (E) and galactose-fed (F) rats. Neither edema nor myelin sheath defects were observed in sural nerves from galactose-fed rats. Peroneal nerves from control (G) and galactose-fed (H) rats. Myelinated fibers are closely spaced in the control, while marked interstitial edema is seen in the peroneal nerve of the galactose-fed rat. Although not illustrated here, myelin splitting and ballooning were also occasionally observed in peroneal nerves from galactose-fed rats. While NT-3 prevented galactose-induced axonal dwindling in the dorsal root and sural nerve, it did not attenuate myelin splitting or edema in any region of the peripheral neuraxis. *p*-phenylenediamine. Bar in (H) = 40 μm.

Previous studies have documented axonal dwindling in experimental diabetes (13, 15, 20, 30, 39–41) and it has been calculated that the reduction in caliber observed is sufficient to account for the decrease in nerve conduction velocity (39). In the present study, myelinated fibers of untreated galactose-fed rats are characterized by a decrease in mean axonal diameter and a shift in axonal size-frequency distribution towards smaller values with a consequent reduction of the relative number of large fibers (Table 3; Figs. 1, 2). The decrease in mean diameter and shift in size-frequency distribution are likely the result of diminution in axonal caliber because, with the exception of the ventral root in this study, galactose intoxication of this duration does not cause axonal degeneration or loss (13, 30).

The mechanism by which NT-3 treatment prevents the galactose-induced deficit in sciatic and saphenous SNCV may involve structural modifications. In parallel with NT-3’s effect on sensory but not motor nerve conduction deficits, NT-3 attenuated reductions in axonal caliber and shifts in size-frequency distribution in some sensory components of the peripheral neuraxis, including the dorsal root and sural nerve, but was without effect in motor components such as the ventral root and peroneal nerve. Decreased axonal caliber of myelinated fibers in galactose-fed rats has been associated with a decrease in the
number of neurofilaments per axon (41), suggesting deficient neurofilament synthesis, assembly, or transport. Alterations in neurofilament H phosphorylation have been reported in the spinal cord of diabetic rats (42). Consequently, it would be of interest to explore the possibility that NT-3 treatment normalizes or even accelerates neurofilament expression in the dorsal root and sural nerve which, by preserving axonal caliber, prevents sciatic SNCV deficits in experimental diabetes.

In contrast to the sciatic nerve and its branches, NT-3 treatment prevented galactose-induced SNCV deficits in the saphenous nerve in the absence of effects on myelinated fiber structure as assessed by axonal diameters (Table 3) and size-frequency distributions (data not shown). The reason for this difference is not clear and reflects an effect of NT-3 treatment on a parameter not measured in the present study. Although axonal caliber is known to be a factor in determining nerve conduction velocity, it may be less important than other structural parameters such as internodal length. In addition, alterations in any of a variety of less widely studied characteristics of nerve fibers, such as composition of axoplasmic fluid (43), conductance and permeability of the axonal membrane (44), and capacitance and insulation provided by the myelin sheath (45–47), could have an impact on conduction velocity in experimental diabetes.

It is clear that exogenous NT-3 does not prevent changes in myelin capacitance induced by splitting and ballooning of the myelin sheath in the dorsal and ventral roots (Table 4). Splitting and ballooning has been reported in the roots of streptozotocin-diabetic (48) and galactose-fed (20) rats, and also in aging (49–51) and neuropathies resulting from intoxication with triethylin (52), hexachlorophene (53), dideoxyctidine (54) and pyridoxine (55). In diabetes and aging, this myelin defect has been suggested to be related to excessive nonenzymatic protein glycation (56), but its presence in triethylin; hexachlorophene, dideoxyctidine and pyridoxine poisoning, conditions not known to produce advanced glycosylation endproducts, points to other possible etiologies including alterations in the expression of myelin structural proteins due to aging, diabetes and/or toxins. In galactose-fed rats, myelin splitting and ballooning is prevented in both the dorsal and ventral root by AR inhibition. The incidence of this defect in the ventral root is significantly attenuated by treatment with BDNF, which prevents the galactose-induced motor, but not sensory, conduction deficit (20).

In summary, subcutaneous administration of exogenous NT-3 prevented the development of conduction deficits in sensory, but not motor, nerves of adult galactose-fed rats in a dose-dependent manner. Specificity for large-fiber sensory components of the peripheral neuraxis was also reflected, in part, by the ability of NT-3 treatment to prevent axonal dwindling in the dorsal root and sural nerve, but not the ventral root and peroneal nerve. Treatment of control animals with NT-3 had no effect on these functional and structural parameters. These data provide an experimental rationale for the use of NT-3 in treatment of the large-fiber component of human diabetic neuropathy.

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