Impaired Prosaposin Secretion During Nerve Regeneration in Diabetic Rats and Protection of Nerve Regeneration by a Prosaposin-Derived Peptide

Corinne G. Jolivalt, PhD, Yvonne Vu, MD, Leah M. Mizisin, BS, Andrew P. Mizisin, PhD, and Nigel A. Calcutt, PhD

Abstract

Prosaposin is both a precursor of sphingolipid activator proteins and a secreted neurotrophic and myelinosorphic factor. Because peripheral nerve regeneration is impaired in diabetes mellitus, we measured prosaposin protein levels from control and streptozotocin-diabetic rats by collecting endoneurial fluid secreted into a bridging tube connecting the ends of transected sciatic nerve. Prosaposin protein levels were significantly reduced in endoneurial fluid from diabetic rats and increased in the proximal nerve stump compared to controls. To investigate whether a prosaposin-derived peptide could improve nerve regeneration, rats were treated with prosaptide TX14(A) after sciatic nerve crush. In control rats, TX14(A) was without effect in the uninjured nerve but shortened toe spread recovery time after nerve crush. In diabetic rats, efficacy of prosaptide TX14(A) was confirmed by correction of thermal hypoalgesia, formalin-evoked hyperalgesia, and conduction slowing in the uninjured nerve. The peptide also prevented diabetes-induced abnormalities in nerve regeneration distance and mean axonal diameter of regenerated axons, whereas delayed recovery of toe spread was not improved. Muscle denervation atrophy was attenuated by TX14(A) in both control and diabetic rats. These results suggest that reduced prosaposin secretion after nerve injury may contribute to impaired regeneration rates in diabetic rats, and that prosaptide TX14(A) can improve aspects of nerve regeneration.

Key Words: Diabetes, Nerve regeneration, Prosaposin, Prosaptide TX14(A).

INTRODUCTION

Peripheral nerve regeneration after physical injury is orchestrated by the sequential expression and release of a series of neurotrophic factors and cytokines from Schwann cells, invading macrophages, and other cells adjacent to the injury site. Axonal survival, sprouting, and maturation are regulated by members of the neurotrophin, glial cell-derived neurotrophic factor, and neuropoietic cytokine families, whereas assorted receptors on Schwann cells and other cellular components of the nerve microenvironment. Together, these systems allow peripheral nerve to reinnervate its original targets and restore function, although the recovery is usually imperfect with features such as internodal distance, axonal caliber, and therefore conduction velocity, being suboptimal compared with the preinjury condition.

It has been argued that the distal symmetric polyneuropathy associated with diabetes mellitus represents an imbalance of neuronal survival and maintenance that is driven by increased distal degeneration and an impaired capacity to initiate and maintain the regenerative responses to injury. The epidermal nerves of diabetic patients exhibit a reduced regenerative capacity after physical trauma, whereas many studies in diabetic rodents have shown that recovery from nerve crush injury is delayed, slowed, or morphologically disrupted. Interventions that can afford neuroprotection and stimulate regeneration have the potential to both shift the balance away from progressive degeneration and also enhance recovery of axons that are damaged but not yet dead.

Prosaposin is the precursor of saposin proteins involved in lysosomal sphingolipid degradation and is also found as a holoprotein in body fluids such as cerebrospinal fluid and milk. Prosaposin is secreted into the endoneurial fluid that accumulates distal to a peripheral nerve transection and induces neuritogenesis in vitro, implicating it as a potential factor involved in nerve regeneration. Prosaptide TX14(A), a modified 14-mer peptide fragment of prosaposin that lacks saposin activity but retains neurotrophic properties, also induces neuritogenesis. Prosaptide TX14(A) exhibits a number of neuroprotective properties in animal models of diabetic neuropathy, including prevention of conduction slowing and axonal atrophy in the peripheral nerve of diabetic rats. Given the neuroprotective properties of prosaptide TX14(A) in diabetic rats and the potential role of prosaposin in peripheral nerve regeneration, we investigated whether the impaired nerve regeneration of diabetic rats is associated with an impediment of prosaposin secretion at the injury site and
whether prosaptide TX14(A) has effects on indices of nerve regeneration in normal and diabetic rats.

**MATERIALS AND METHODS**

**Animals**

All experiments were performed on adult (starting weight, 220–240 g) female Sprague-Dawley rats (Harlan Industries, San Diego, CA) using procedures approved by the local Institutional Animal Care and Use Committee. Rats were allowed free access to food and water and were maintained on paper bedding with a 12-hour light-dark cycle.

Rats were made diabetic by the injection of streptozotocin (55 mg/kg, intraperitoneal [i.p.], in saline) after an overnight fast. Hyperglycemia was confirmed 4 days later by measuring glucose levels in a drop of tail vein blood using a strip-operated reflectance meter and at the end of the study by spectrophotometric assay (glucose assay kit; Sigma, St. Louis, MO). Only rats with a blood sugar of 15 mmol/L or higher were accepted as being diabetic.

**Prosaposin Secretion**

After 12 weeks of diabetes, rats were anesthetized using a mixture of sodium pentobarbital (12.5 mg/kg) and diazepam (1.25 mg/ml) in physiologic saline (2 ml/kg, i.p.), the hindquarters were shaved, and the surgery area was prepared with Betadine. Both sciatic nerves were exposed by a lateral incision in the thigh, and the overlying muscles were separated. Nerves were mobilized from the sciatic notch to the tibial-peroneal bifurcation, and a 1-cm segment at the midheight level was resected and stored at −70°C. The proximal and distal stumps were sutured into the open ends of a 14-mm-long silicon tube with 7-0 silk suture passing through the epineurium, so that the resulting gap between the severed stumps was 10-mm long. One or 9 days after nerve transection, animals were reanesthetized, both sciatic nerves were removed, and both tubular fluid and 1 cm of proximal stump from both tubes of each animal were collected. Nerve samples were homogenized into 200 μl of ice-cold homogenization buffer (50 mmol/L of Tris-HCl, pH 7.4, 150 mmol/L of NaCl, 0.5% Triton X, 1 mmol/L of EDTA, 4 μl/ml of protease inhibitor cocktail) and centrifuged at 14,000 × g for 30 minutes at 4°C. After determination of sample protein concentration (BCA protein assay; Pierce, Rockford, IL), 4 μg of total extracted protein or 1.5 μl of tubular fluid samples diluted 30 times in PBS was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 12% Bis-Tris gel (Invitrogen Life Technologies, Carlsbad, CA). Proteins were electrotransferred onto a nitrocellulose membrane (Hydron-C extra; Amersham Life Science, Arlington Heights, IL) and then incubated in 3% bovine serum albumin/PBS containing 0.05% (vol/vol) Tween 20. Membranes were immunostained with an affinity-purified anti-saposin C antibody (1:10000; GenWay Biotech, Inc., San Diego, CA) or β-actin (1:2000; Sigma). Blots were washed 3 times for 10 minutes with PBS containing 0.05% (vol/vol) Tween 20, followed by a secondary anti-chicken antibody (1:20000; Sigma) or an anti-mouse antibody (1:10000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After 3 further washes in PBS containing 0.05% (vol/vol) Tween 20, immunodetection was performed using enhanced chemiluminescence (ECL kit; Amersham). For repeated analysis of membranes, previously bound antibodies were removed using stripping buffer (0.2 mol/L of glycine, pH 2.5, 0.05% Tween 20) for 45 minutes at 60°C. After immunostaining, the amount of prosaposin was determined densitometrically using Quantity One software. Sciatic nerve samples were normalized by calculating the ratio of the intensity of the band corresponding to prosaposin to the intensity of the band corresponding to actin. Prosaposin protein in endoneurial fluid was normalized to fluid volume.

**Nerve Regeneration**

Two methods were used to assess nerve regeneration after injury, a direct measure of neuronal regeneration distance and an indirect measure by monitoring recovery of limb function. We have previously used both techniques to demonstrate that diabetes impedes nerve regeneration and limb functional recovery (10). Briefly, both sciatic nerves were exposed under anesthesia, as previously described, and 1 sciatic nerve was crushed for 5 seconds at the level of the obturator tendon using watchmaker’s forcesps, with the crush site marked by an epineurial suture. The contralateral nerve was mobilized, but not crushed, and served as a sham-operated control. After nerve crush, the skin incision was closed, and the animal was allowed to recover. Nerve regeneration was allowed to proceed for 3, 7, or 32 days. Animals assigned to the 3- or 7-day regeneration time points were anesthetized, and the crushed sciatic nerve was re-exposed. Nerve regeneration distance was measured using a stimulating electrode (5 V, 0.05-millisecond width, 0.5-Hz pulses from a Grass stimulator) initially placed to touch the nerve 30 mm distal to the crush site and then advanced in 0.5-mm increments toward the crush site until a reflex response was recorded in the musculature of the back. The distance from the stimulation point to the crush site was measured with calipers. Regeneration was measured in the animals assigned to the 32-day time point using recovery of toe spread in the injured limb. Both hind paws were coated with Betadine, and the animal was encouraged to walk across paper, with subsequent measurement of the distance between prints of the first and fifth toe of the injured and uninjured paw. Three prints of each paw were measured at each time point, and the median toe spread was calculated. Toe spread analysis was performed both before, and on a regular basis after, nerve injury and change in toe spread calculated at each time point relative to preinjury values for each paw.

**Other Assays**

At the conclusion of the study, rats were anesthetized with halothane and nerve conduction velocity measured in both the sham-operated and injured sciatic nerves using stimulating electrodes (5–10 V, 0.05-millisecond pulse width) placed at the sciatic notch and Achilles tendon and recording electrodes placed in the ipsilateral interosseous muscles. Nerve
temperature was maintained at 37°C using a heating lamp. Resulting M and H waves were recorded on a digital storage oscilloscope, and conduction velocity was calculated as the distance between the stimulation sites divided by the time difference between the M or H wave peaks obtained from each stimulation site. Measurements were made in triplicate for each nerve and the median used to represent motor and sensory conduction velocity (MNCV and SNCV) for that nerve. Paired SEM. Between-group comparisons were made either by unpaired t-test or by 1-way analysis of variance with post hoc analysis using the Student-Newman-Keuls test to compare all groups or Dunnett test to selectively identify groups that were significantly different from the vehicle-treated control or the vehicle-treated diabetic group.

**RESULTS**

**Nerve Prosaposin Content and Secretion After Injury**

The prosaposin/saposin C antibody identified a single protein band at 72 kDa (Fig. 1), which is consistent with the molecular weight of whole prosaposin. No bands were detected at 12 to 16 kDa, the molecular weight of saposins (data not shown). Prosaposin content in the portion of sciatic nerve that was excised before implanting the connecting tube was similar in control and diabetic rats after 4 weeks of hyperglycemia (Fig. 1). The prosaposin content of the sciatic nerve immediately proximal to the bridging tube was markedly lower than that of the excised nerve (labeled “before”) on Days 1 and 9 after transection in both control and diabetic rats (Fig. 2). Prosaposin protein content of this proximal nerve segment was similar between control and diabetic rats on Day 1 after transection, but by Day 9, this proximal nerve segment contained significantly (p < 0.01) less prosaposin protein in control rats than in diabetic rats (Fig. 2). In contrast, the prosaposin content of endoneurial fluid collected from the tube connecting the proximal and distal stumps was significantly (p < 0.05) greater in control rats compared with diabetic rats on both Days 1 and 9 (Fig. 3).

**Effects of Prosaptide TX14(A) on Rate of Nerve Regeneration**

Six animals per group were studied on Days 3 and 7 after crush injury. Vehicle-treated diabetic rats exhibited
weight loss (209 ± 5 g; mean ± SEM) and hyperglycemia (30.0 ± 2.0 mmol/L) compared with vehicle-treated controls (242 ± 2 g and 7.1 ± 0.4 mmol/L). Prosaptide TX14(A) did not affect these parameters in either control (224 ± 3 g and 7.0 ± 0.6 mmol/L) or diabetic (195 ± 4 g and 34.5 ± 1.2 mmol/L) rats. Vehicle-treated diabetic rats showed a significantly (p < 0.05) reduced distance of regeneration compared with vehicle-treated controls 3 days after crush injury, and a similar deficit was observed in diabetic rats treated with prosaptide TX14(A) (p < 0.05; Fig. 4). Nerve regeneration distance continued to be significantly (p < 0.05) reduced on Day 7 after crush injury in vehicle-treated diabetic rats, but by this time, the diabetic rats treated with TX14(A) showed a regeneration distance that was significantly (p < 0.05) higher than the vehicle-treated diabetic rats and not significantly different from control rats (Fig. 4). Prosaptide TX14(A) significantly (p < 0.05) slowed nerve regeneration distance in control rats on Day 3, but not Day 7, after injury (Fig. 4).

Effects of Prosaptide TX14(A) on Nerve and Limb Function in Diabetes and After Crush Injury

Eight animals per group were followed for 4 weeks of diabetes and then 4 more weeks after sciatic nerve crush injury. After 8 weeks of diabetes, rats showed hyperglycemia and reduced body weight compared with vehicle-treated controls, and treatment with prosaptide TX14(A) for the last 4 weeks did not alter these parameters in either control or diabetic rats (Table 1). Measurements made in the uninjured limb 48 hours after the last treatment with vehicle or prosaptide TX14(A) demonstrated that diabetes induced significant MNCV and SNCV slowing, thermal hypoalgesia, and hyperalgesic behavior during Phase 2 of the response to 0.5% paw formalin injection. Treatment with TX14(A) for the last 4 weeks of the study did not significantly alter any of these parameters in control rats. The uninjured limb of diabetic rats treated with prosaptide TX14(A) showed significant alleviation of MNCV and SNCV slowing, thermal hypoalgesia, and
The EDL muscle weight in the uninjured limb of control rats was significantly (p < 0.001) higher than that of diabetic rats, reflecting diabetes-induced muscle wasting (Table 2). Prosaptide TX14(A) treatment did not influence EDL muscle weight in either control or diabetic rats. The weight of the EDL muscle in the nerve-injured limb was markedly lower than that of the contralateral uninjured limb in all groups, suggesting denervation atrophy. The decline in muscle weight, calculated as the EDL muscle weight as a percentage of the body weight in either control or diabetic rats, reflecting diabetes-induced muscle wasting (Table 2).

Motor nerve conduction velocity in the regenerated nerve of either control or diabetic rats, whereas prosaptide TX14(A) did not affect MAD of control rats (Fig. 5; Table 2).

Before nerve crush or the onset of treatment, the toe-spread distance of control rats (21.9 ± 0.4 mm; cohort mean ± SEM) was significantly higher than that of diabetic rats (18.1 ± 0.5 mm; p < 0.01 by unpaired t-test). After crush injury, the toe-spread distance in the injured limb declined relative to preinjury values in all groups (Fig. 6). In vehicle-treated control rats, recovery of toe spread occurred between Days 26 and 28, whereas in control rats treated with prosaptide TX14(A), recovery was already clear by Day 24 (p < 0.05 vs all other groups by 1-way analysis of variance, followed by Student-Newman-Keuls post hoc test). Vehicle-treated diabetic rats did not show complete recovery of toe spread during the 32 days of the study, and a similar pattern was seen in diabetic rats treated with prosaptide TX14(A) such that on Day 32, both diabetic groups were significantly different from both control groups (p < 0.05 by 1-way analysis of variance, followed by Student-Newman-Keuls post hoc test).

**Effects of Prosaptide TX14(A) on Nerve Prosaposin Expression**

We measured nerve prosaposin content in sciatic nerve samples taken from control rats, rats after 8 weeks of untreated

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**TABLE 1.** Effect of Prosaptide TX14(A) on General Physiology and Uninjured Nerve Function After Treatment

<table>
<thead>
<tr>
<th>Glucose, mmol/L</th>
<th>Body Weight, g</th>
<th>Sciatric MNCV, m/s</th>
<th>Sciatric SNVC, m/s</th>
<th>Thermal Latency, s</th>
<th>0.5% Formalin Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.1 ± 0.3b</td>
<td>242 ± 2b</td>
<td>60.1 ± 1.8b</td>
<td>9.6 ± 1.0b</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Control + TX14(A)</td>
<td>7.0 ± 0.2b</td>
<td>245 ± 3b</td>
<td>62.3 ± 1.4b</td>
<td>11.2 ± 0.9b</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Diabetic</td>
<td>34.7 ± 2.9b</td>
<td>209 ± 5b</td>
<td>50.3 ± 0.9b</td>
<td>15.9 ± 1.9b</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Diabetic + TX14(A)</td>
<td>33.5 ± 2.2b</td>
<td>195 ± 9b</td>
<td>57.9 ± 1.3b</td>
<td>8.2 ± 0.6b</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

*Statistical significance: a versus b, p < 0.01; a versus c, p < 0.05.

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**TABLE 2.** Effects of Diabetes and Prosaptide TX14(A) on Responses of Muscle and Nerve to Nerve Crush Injury

<table>
<thead>
<tr>
<th>Uninjured, g</th>
<th>EDL Muscle</th>
<th>Atrophy, %</th>
<th>Regenerated Nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134.5 ± 6.8a</td>
<td>101.2 ± 4.9a</td>
<td>75.3 ± 1.6a</td>
</tr>
<tr>
<td>Control + TX14(A)</td>
<td>129.7 ± 3.4a</td>
<td>106.1 ± 2.5a</td>
<td>81.7 ± 1.7b</td>
</tr>
<tr>
<td>Diabetic</td>
<td>64.0 ± 3.5b</td>
<td>42.8 ± 2.3b</td>
<td>66.4 ± 1.9b</td>
</tr>
<tr>
<td>Diabetic + TX14(A)</td>
<td>65.2 ± 4.7b</td>
<td>47.7 ± 4.4b</td>
<td>72.6 ± 2.3b</td>
</tr>
</tbody>
</table>

*Statistical significance: a versus b, p < 0.001; a versus c, p < 0.001; a versus d, and a versus b, p < 0.05.

*Data are mean ± SEM. Statistical analyses by 1-way ANOVA, followed by the Student-Newman-Keuls post hoc test.

ANOVA, analysis of variance; MNCC, motor nerve conduction velocity; SEM, standard error of mean.
streptozotocin-diabetes, and rats treated with 1 mg/kg of prosaptide TX14(A) thrice weekly for 8 weeks of streptozotocin-induced diabetes to determine whether the effects of prosaptide TX14(A) on nerve regeneration were mediated by induction of nerve prosaposin expression. There was no significant difference in nerve prosaposin content between either of the 2 diabetic groups and the control group (untreated diabetic, 105 ± 9; treated diabetic, 103 ± 7; prosaposin per lane calculated relative to actin loading and group data calculated as percentage ± SEM of mean control values present on the same gel; n = 7 per group). The impact of diabetes and efficacy of the prosaptide TX14(A) treatment regime on the biochemistry,

FIGURE 5. (A) Axonal size: frequency distribution in the sciatic nerve distal to the crush site measured 1 month after the injury in control rats (gray area under curve), control rats treated with prosaptide TX14(A) after crush injury (filled circles), diabetic rats (line without markers), and diabetic rats treated with prosaptide TX14(A) after crush injury (filled diamonds). Data are group mean ± standard error of mean for each size bin. (B) Light microscopic images illustrating nerve distal to the crush site from control and diabetic rats used for morphometric analysis. Bar = 60 μm.

FIGURE 6. (A) Toe spread in the nerve-injured limb of (A) vehicle-treated control rats (black circles) and prosaptide TX14(A)-treated control rats (white circles) and (B) vehicle-treated diabetic rats (black squares) and TX14(A)-treated diabetic rats (white squares). Data are group mean ± standard error of mean (n = 8 per group). Statistical analysis was performed at Days 24 and 32 and incorporated all 4 groups (see text for details).
DISCUSSION

Prosaposin is the precursor for lysosomal saposins, but within the nervous system, the holoprotein is the dominant form (12). Whole prosaposin is localized to neuronal cell bodies of the CNS and secreted into cerebrospinal fluid (12, 13). Prosaposin protein is also found in sensory cell bodies of the peripheral nervous system, and prosaposin mRNA is detected in nonneuronal cells within peripheral nerve trunks (8). An association with nerve repair processes is suggested by reports that prosaposin mRNA increases in the distal stump after nerve transaction (14), whereas prosaposin protein is detected in the endoneurial fluid that bridges the transected nerve (4). Cell culture studies demonstrate that exogenous prosaposin induces neuritogenesis (5, 7), and that it promotes both the myelinating phenotype of Schwann cells (4, 15) and maturation of muscle (16). Addition of prosaposin into the region between proximal and distal stumps of a transected nerve dose-dependently increased the number of regenerating axonal sprouts (17). These data suggest that secreted whole prosaposin may have a role in the recovery of peripheral nerve and muscle after injury.

We measured nerve prosaposin protein levels using an anti-saposin C antibody. The 72-kDa molecular weight of the sole protein band detected with this antibody is consistent with that of whole prosaposin, rather than saposin C (12–16 kDa), confirming a recent report that unprocessed prosaposin is the dominant form in the peripheral nervous system (12). The relative decline of prosaposin protein in the proximal stump after transection compared with levels in uninjured nerve is consistent with decreased prosaposin protein in neuronal cell bodies of the facial nerve on Days 7 and 14 posttransection (13), although the axonal transport dynamics of prosaposin in the peripheral nervous system is not yet known. In contrast, prosaposin mRNA increases in the distal stump after nerve injury, implying injury-induced upregulation of subsequent prosaposin synthesis by nonneuronal cells (14). It will be important to determine whether there is a parallel upregulation of prosaposin mRNA and protein expression in the proximal stump and also the relative contributions of neuronal and nonneuronal prosaposin in the proximal stump to clarify the sources of prosaposin in the endoneurial fluid.

We also tested the hypothesis that the impaired nerve regeneration of diabetic rats (3, 13) is associated with reduced prosaposin availability. Neither 8 nor 12 weeks of diabetes altered the amount of prosaposin protein in the uninjured sciatic nerve. Prosaposin mRNA is increased in the sciatic nerve of rats after 8 weeks of diabetes (8), indicating that the 2-fold increase in mRNA does not result in a parallel elevation of protein. This apparent dichotomy could reflect diabetes-induced inefficiency in translation because amino acid uptake into nerve from diabetic rats is reduced (18) or, alternatively, that cells containing prosaposin mRNA in peripheral nerve (8) contribute only a small fraction of the measured prosaposin protein. An increase in the rate of prosaposin cleavage into lysosomal saposins is a less likely explanation because we did not detect saposin C in the nerve or endoneurial fluid.

Prosaposin protein in the proximal stump of diabetic rats initially declined in parallel with the protein in control rats, but this decline did not progress, so that there was significantly more prosaposin protein in the proximal stump of the sciatic nerve from diabetic rats compared with controls. Conversely, prosaposin protein levels in the endoneurial fluid collected beyond the point of transection were lower in diabetic rats compared with controls. One interpretation of these findings is that diabetes impedes secretion of neuronal prosaposin from the proximal stump so that it accumulates in the axon terminals and does not appear in the endoneurial fluid. The description of swollen and dystrophic regenerating axons projecting into the bridging tube of diabetic rats (19) may support this speculation. The impact of diabetes on prosaposin synthesis, anterograde axonal transport, and turnaround at terminals by diabetic neurons requires further evaluation, however, before the attenuated decline of prosaposin in the proximal stump can be fully addressed. The contribution of prosaposin secreted from nonneuronal sources in both proximal and distal stumps must also be measured to clarify the cause of the diabetes-induced diminution of prosaposin in endoneurial fluid. Nevertheless, because prosaposin promotes neuritogenesis and neurite extension in cultured neurons and peripheral nerve (5, 17), the reduction of prosaposin in the endoneurial fluid through which regeneration takes place is consistent with the impaired rates and quality of regeneration seen in diabetic rats after nerve transection and regrowth through a similar bridging tube (19–21).

Prosaptide TX14(A) is a 14-mer peptide analog of the neurotrophic region of the saposin C domain of prosaposin (5). Like whole prosaposin, prosaptide TX14(A) induces neurite growth in transformed and adult primary sensory neurons that model neuronal regeneration after injury (6). Therefore, we investigated whether systemic prosaptide TX14(A) enhanced nerve regeneration and limb recovery after nerve crush injury. In control rats, the peptide was without effect on nerve function in the uninjured limb or indices of axonal growth and maturation in the injured nerve. It did, however, accelerate the time to recovery of limb function as measured by toe spread after nerve crush injury. This measurement likely represents a combination of nerve regeneration rate, which was unaffected by prosaptide up to 7 days after injury, and by subsequent formation of functional neuromuscular synapses. The efficacy of prosaptide TX14(A) may therefore derive from enhanced motor end-plate sprouting, as occurs after local injection of the peptide to muscle (6). The accelerated recovery of toe spread in control rats was accompanied by mitigation of EDL muscle atrophy in the ipsilateral limb, with the latter potentially being a consequence of the former. Alternately, because prosaptide TX14(A) also attenuates atrophy of the EDL muscle after sciatic nerve ligation, a paradigm that does not allow reinnervation (16), the peptide may have a direct effect on the response of muscle to nerve injury that is independent of nerve regenerative capacity.

Systemic delivery of prosaptide TX14(A) protects peripheral nerve from diabetes-induced disorders of nerve
structure and function (8, 9, 22, 23). Similar effects were seen in the present study, confirming the efficacy of the treatment regime without any reduction in the severity of diabetes. The peptide also prevented both the slowed nerve regeneration at 7 days postcrush and the increased MAD of regenerating axons measured 1 month after crush injury seen in diabetic rats. An increase in larger diameter axons has previously been reported in regenerating diabetic nerve after transection and was attributed to dystrophic accumulations (19) or a decrease in the relative proportion of smaller axons (20). Qualitative electron microscopic evaluation did not show any consistent occurrence of dystrophic accumulations of either organelles or cytoskeletal elements in the regenerating axons of diabetic rats (A.P. Mizisin, data not shown). Moreover, despite maintaining normal rates of regeneration over the first 7 days after injury in diabetic rats, prosaptide TX14(A) did not prevent the failure of diabetic rats to recover toe spread distance. This may be because the efficacy of prosaptide TX14(A) in maintaining nerve regeneration rates in diabetic rats diminishes after Day 7 or reflects the contribution of disorders other than nerve regeneration rate to recovery of toe spread that is not protected by the peptide. The capacity of prosaptide TX14(A) to partially protect EDL muscle weight of diabetic rats (A.P. Mizisin, data not shown). Moreover, the peptide also prevented both the slowed nerve regeneration at 7 days postcrush and the increased MAD of regenerating sprouts or at the cell body, as is seen in vitro (6). The initial site of action of systemically delivered prosaptide TX14(A) is not known, however, and indirect effects of the peptide that lead to enhanced nerve regeneration cannot be discounted. Prosaptide TX14(A) did not induce prosaposin protein in nerve of diabetic rats, but other agents with neurotrophic properties have been reported to improve indices of nerve regeneration after injury; these include ciliary neurotrophic factor (10), insulin (31), C peptide (32), insulinlike growth factor (33), nerve growth factor (21), and gangliosides (34, 35). It remains plausible that prosaptide TX14(A) induces 1 or more of these factors in nerve or other tissues. In summary, our studies show that impaired nerve regeneration in diabetic rats is accompanied by the reduced appearance of prosaposin in the environment through which regenerating axons grow. A peptide analog of prosaposin that shares neuritogenic properties with the holoprotein enhanced recovery of limb function in control rats and prevented indices of degenerative neuropathy and slowed regeneration in diabetic rats. Because diabetic neuropathy is characterized by both distal degeneration and an impaired regenerative capacity, prosaptide TX14(A) may both protect nerve from damage during diabetes and promote the recovery of damaged nerves.

REFERENCES