Insulin-like Growth Factor I Reverses Experimental Diabetic Autonomic Neuropathy

Robert E. Schmidt,* Denise A. Dorsey,*
Lucie N. Beaudet,* Santiago B. Plurad,*
Curtis A. Parvin,[†] and Matthew S. Miller[‡]

From the Divisions of Neuropathology* and Laboratory Medicine,[†] Department of Pathology, Washington University School of Medicine, St. Louis, Missouri; and Cephalon, Inc.,[‡] West Chester, Pennsylvania

Recent studies have suggested a role for neurotrophic substances in the pathogenesis and treatment of diabetic neuropathy. In this study, the effect of insulin-like growth factor I (IGF-I) on diabetic sympathetic autonomic neuropathy was examined in an experimental streptozotocin-induced diabetic rat model. Two months of IGF-I treatment of chronically diabetic rats with established neuroaxonal dystrophy (the neuropathological hallmark of the disease) involving the superior mesenteric ganglion and ileal mesenteric nerves resulted in nearly complete normalization of the frequency of neuroaxonal dystrophy in both sites without altering the severity of diabetes. Treatment with low-dose insulin (to control for the transient glucose-lowering effects of IGF-I) failed to affect the frequency of ganglionic or mesenteric nerve neuroaxonal dystrophy or the severity of diabetes. The striking improvement in the severity of diabetic autonomic neuropathy shown with IGF-I treatment in these studies and the fidelity of the rat model to findings in diabetic human sympathetic ganglia provide promise for the development of new clinical therapeutic strategies. (Am J Pathol 1999, 155:1651–1660)

Peripheral nervous system dysfunction is a significant complication of both types 1 (insulin-dependent diabetes mellitus, IDDM) and 2 (non-insulin-dependent diabetes mellitus, NIDDM) diabetes, affecting ~60% of all diabetics.¹ The most common and best studied clinical presentation of diabetic neuropathy is as a symmetrical sensorimotor neuropathy, resulting in classical “stocking-glove” limb anesthesia. However, other patterns of diabetes-induced neuropathy include asymmetrical neuropathies, ie, single or multiple mononeuropathies involving cranial and somatic nerves, and autonomic neuropathy.² Autonomic neuropathy in diabetes is characterized by symptoms ranging widely from minor pupillary and sweating problems to significant disturbances in cardiovascular, alimentary, and genitourinary function³ and results in increased patient mor-

bidity and mortality.^{4,5} Although early studies stressed preferential parasympathetic involvement in diabetic autonomic neuropathy, recent power spectral analysis, m[¹²³I]iodobenzylguanidine (MIBG) cardiac imaging studies, and microneurographic studies of muscle sympathetic activity have established an early significant sympathetic component, which may precede parasympathetic dysfunction.^{6,7}

The detailed pathogenesis of peripheral nervous system dysfunction in diabetes is unknown; indeed, multiple mechanisms may participate interactively and may vary between different forms of diabetic neuropathy. Proposed pathogenetic mechanisms (reviewed in refs 8 and 9) have, in large part, been based on clinical and animal studies of somatic nerves and myelinated axons. A variety of metabolic alterations (eg, increased activity of the polyol pathway, phosphoinositide dysmetabolism, glycation processes, and increased oxidative stress)^{10–12} may result secondarily in ischemia,¹³ interfere with the structure or function of the axonal cytoskeleton,¹⁴ induce autoimmune-mediated damage,¹⁵ or result in the deficiency of various neurotrophic substances.^{16–20}

Neurotrophic substances have been implicated in the pathogenesis and/or treatment of diabetic neuropathy as well as a variety of toxic, traumatic, metabolic, and hereditary neuropathies with defects proposed or identified variously in their circulating levels or end organ production, nerve terminal binding, or axonal transport.^{21,22} A role for nerve growth factor (NGF), in particular, in the pathogenesis of diabetic somatic sensory and autonomic neuropathies has been supported by the demonstration of decreased axonal transport of labeled and endogenous NGF in somatic and autonomic nerves,^{16,17} decreased dorsal root ganglion (DRG) and superior cervical ganglion (SCG) ganglionic NGF content,^{18,23} and reduced expression of NGF-sensitive sensory neuronal neuropeptides CGRP and substance P in the sciatic nerve (which may be ameliorated with exogenous NGF administration).^{23,24} However, other data suggest that sympathetic autonomic neuropathy may not simply reflect a NGF deficiency state in diabetes. There is neither significant loss of neurons nor a decrease in the size of

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Address reprint requests to Dr. Robert E. Schmidt, Department of Pathology, Division of Neuropathology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110. E-mail: reschmidt@pathology.wustl.edu.

their perikarya in the paravertebral SCG²⁵ or prevertebral superior mesenteric ganglion (SMG)¹⁷ sympathetic ganglia of diabetic rats, as would be expected in pathophysiologically significant NGF deprivation. Second, the activity of the NGF-sensitive enzyme tyrosine hydroxylase²⁶ is unchanged (SCG) or substantially increased (SMG) in long-term diabetics. Finally, neither SMG nor SCG neurons develop the characteristic neuropathology of diabetic autonomic neuropathy in response to simple autoimmune NGF deprivation in nondiabetic rats.²⁷

A role for insulin-like growth factor I (IGF-I) and/or insulin-like growth factor II (IGF-II), neurotrophic substances that bind to specific cellular receptors and, thereby, support sensory and sympathetic neurons in culture,^{28,29} has also been proposed³⁰ for the pathogenesis of somatic neuropathy in diabetic rats. In support of this hypothesis, expression of IGF-I and IGF-II genes is reduced in the spinal cord and Schwann cells of the sciatic nerves of rats with experimental streptozotocin (STZ)-induced diabetes,^{31,32} and circulating levels of serum IGF-I are also diminished in this model.³³ Depressed somatic nerve conduction velocity, which is a characteristic finding in rats with experimental STZ diabetes, has been corrected unilaterally by near-nerve application of subhypoglycemic doses of insulin.³⁴ Similarly, diabetes-induced hyperalgesia involving rat somatic nerves is largely corrected by exogenously administered IGF-I.³² In addition, failure to properly up-regulate IGF-I in the distal axonal segment in response to sciatic nerve axotomy^{30,35} may underlie the identified defect in somatic axonal regeneration in diabetic rats,^{36,37} a defect ameliorated by exogenously administered IGF-I.³² Diabetic rats also show increased levels of IGFBP-1, one of several IGF-I binding proteins, which may sequester IGF-I, reducing its activity, or make it locally available.³⁰ Serum IGF-I levels are decreased in diabetic human subjects with sensory and autonomic neuropathy in comparison to nonneuropathic diabetics or nondiabetic controls.³⁸ Little is known, however, of the effect of IGF-I on the pathogenesis or therapy of diabetic autonomic neuropathy.

To investigate the pathogenesis of diabetic sympathetic autonomic neuropathy, we have developed an animal model^{25,39} and validated it with studies of sympathetic ganglia from a series of autopsied diabetic human subjects,^{40,41} which have shown significant neuropathological similarities. The regular occurrence of degenerating, regenerating, and pathologically distinctive dystrophic axons and synapses has been demonstrated in sympathetic prevertebral celiac ganglia and SMG (but not comparably in the paravertebral SCG)²⁵ and in postganglionic noradrenergic axons distributed to the alimentary tract of rats with chronic long-term STZ-induced diabetes and genetically diabetic BB rats.⁴² Other studies with the STZ-diabetic rat model have also established the time course of the development of neuroaxonal dystrophy,²⁵ characterized its anatomical distribution,^{25,43} determined its relationship to axonal length,²⁵ and examined its response to pancreatic islet cell transplantation,⁴⁴ short or long-term insulin therapy,⁴⁵ and aldose reductase inhibitors.⁴⁶ These pathological findings may underlie altered noradrenergic transmission and ab-

normal gut motility, which have been described in streptozotocin-diabetic rats.^{47,48} Therefore, to identify a possible role of IGF-I in the treatment of diabetic autonomic neuropathy (and, eventually, in its pathogenesis), in this study we have examined the effect of exogenous administration of IGF-I on the frequency of established neuroaxonal dystrophy in our experimental model of diabetic sympathetic autonomic neuropathy.

Materials and Methods

Animals

Male Sprague-Dawley rats (300–350 g) were purchased from Charles River Co. (Belmont, MA) and were housed and cared for in accordance with the guidelines of the Washington University Committee for the Humane Care of Laboratory Animals and with National Institutes of Health guidelines on laboratory animal welfare. Rats were allowed standard rat chow and water *ad libitum* and maintained on a 12/12 hour light/dark cycle.

Animal Protocol

Five groups of rats are represented in this study:

1. untreated age-matched nondiabetic controls ("Controls")
2. untreated diabetics, killed 6 months after the onset of diabetes (ie, at the time at which treatment was initiated, "Diabetic, Pre-Rx")
3. untreated diabetics, killed 8 months after the induction of diabetes ("Diabetic, No Rx")
4. diabetics treated with recombinant human IGF-I (1 mg/kg/day, subcutaneously as a single daily injection; provided by Cephalon, West Chester, PA) for 8 weeks beginning at 6 months of diabetes ("Diabetic + IGF-I")
5. diabetics treated with a small dose of regular recombinant human insulin (humulin, 0.3 U/kg/day, subcutaneously as a single daily injection; Lilly) for 8 weeks, also beginning at 6 months of diabetes ("Diabetic + Humulin"). The group of diabetic animals treated with a low daily dose of humulin represents a control for the small transient effect of IGF-I on blood glucose (a decrease of 70–100 mg/dl for 1–2 hours; P. Contreras, Cephalon, unpublished studies).

Animals were made diabetic by the administration of a single dose of streptozotocin (65 mg/kg in citrate saline buffer, pH 4.5, i.v.; Upjohn, Kalamazoo, MI). Three days after STZ-injection rats were bled, and significantly hyperglycemic animals (plasma glucose > 350 mg/dl) were considered diabetic. Nonfasting morning plasma glucose levels were determined at intervals, and body weights were determined weekly during the 2-month treatment period. Glycated hemoglobin (Glycogel B kit; Pierce, Rockford, IL) was determined at the time of sacrifice.

Table 1. Effect of IGF-I and Low-Dose Humulin on Metabolic Parameters

	Serum glucose (mg/dl)	HbA1c (%)	Final body weight (g)
Control (<i>n</i> = 7)	190 ± 9	4.6 ± .16	460 ± 22
Diabetic, Pre-Rx (<i>n</i> = 6)	431 ± 19*	12.6 ± .6*	378 ± 17 [†]
Diabetic, No Rx (<i>n</i> = 9–11)	427 ± 30*	18.4 ± .96*	254 ± 18*
Diabetic + IGF-I (<i>n</i> = 5–8)	418 ± 17*	19.2 ± .6 [‡]	296 ± 23*
Diabetic + Humulin (<i>n</i> = 7–8)	447 ± 18*	18.3 ± 9 [‡]	256 ± 17*

Statistical comparison: **P* ≤ 0.0001; [†]*P* ≤ 0.01 versus control group; [‡]*P* ≤ 0.0001 versus diabetic, pre-Rx group.

Tissue Preparation

Animals were anesthetized and perfused with 50 ml of heparinized saline followed by 100–200 ml of 3% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3) containing 0.45 mmol/L Ca²⁺. The SMG and ileal mesenteric nerves were dissected free of adjacent tissue and fixed overnight in the same buffer. Tissue samples were post-fixed in phosphate-buffered 2% OsO₄ containing 1.5% potassium ferricyanide, dehydrated in graded concentrations of alcohol, and embedded in Epon with propylene oxide as an intermediary solvent. One-micrometer-thick plastic sections were examined by light microscopy after staining with toluidine blue. Ultrathin sections of individual ileal mesenteric pedicles or SMG were cut onto formvar-coated slot grids, which permits visualization of an entire ganglionic cross section or ileal mesenteric neurovascular pedicle. Tissues were subsequently stained with uranyl acetate and lead citrate and examined with a JEOL 1200 electron microscope.

Quantitative Histological Methods

Sympathetic Ganglia

In our initial quantitative studies²⁵ we expressed the frequency of ganglionic neuroaxonal dystrophy as numbers of dystrophic axons per mm² of ganglionic cross-sectional area. This measurement is potentially subject to variation from one area to another in any individual ganglion and to changes in overall ganglionic size and neuronal density induced by significant neuronal atrophy or loss. In our current study, therefore, we have addressed this potential problem by using a quantitative method we initially developed for determination of the frequency of neuroaxonal dystrophy in human prevertebral superior mesenteric ganglia⁴¹ and have recently used in our studies of diabetic rat sympathetic ganglia.⁴⁹ Dystrophic elements are typically intimately related to neuronal perikarya, and therefore we expressed their frequency as the ratio of numbers of lesions to nucleated neuronal cell bodies. This method substantively reduced the variance in assessments of intraganglionic lesion frequency, and its simplicity permits the quantitative ultrastructural examination of relatively large numbers of ganglia.

In our current animal studies an entire cross section of the SMG was scanned at ×12,000 magnification, and the number of dystrophic axons and synapses was determined. The number of nucleated neurons (range 50–200 neurons examined in each ganglionic cross section) was then determined by recounting at ×6000 magnification.

The frequency of ganglionic neuroaxonal dystrophy was expressed as the ratio of the number of dystrophic axons to the number of nucleated neurons in the same cross section.

Ileal Mesenteric Nerves

Cross sections of three plastic-embedded mesenteric pedicles (ie, neurovascular arcades) sampled approximately 3–5 mm from the gut wall of the last 10 cm of the ileum were examined ultrastructurally in all treated and untreated control and diabetic animals. Because of the anatomy of the mesenteric neurovascular pedicles serving the distal ileum, the sampled individual mesenteric nerves vary in length by only 3–4 mm over the ileal gut segment sampled (in nerves that are more than 120 mm in total length measured from their ganglionic origin). Each mesenteric pedicle contained two (>80% of the time) paravascular nerve fascicles, each approximately 50 μm in diameter and containing 200–500 unmyelinated axons. Ultrathin sections (typically one to three sections/block) were used to identify both paravascular mesenteric nerve fascicles, which were photographed at low magnification for the determination of axon number/fascicle; the number of dystrophic axons in each fascicle was then determined by scanning at ×5000 magnification. The number of dystrophic axons and the total axon number were determined for each of the mesenteric nerve fascicles in three pedicles of each rat. The frequency of dystrophic axons was expressed as a mean value for each animal of the absolute numbers of dystrophic axons per fascicle and as a percentage of the total number of mesenteric nerve axons in the sampled fascicles.

Statistical Analysis

All results are expressed as mean ± SEM. Analysis of variance (ANOVA) was performed with the SAS general linear models procedure.⁵⁰

Results

Metabolic Parameters

Diabetic animals exhibited marked hyperglycemia, decreased body weight, and significantly increased levels of glycosylated hemoglobin compared with age-matched controls (Table 1). Neither IGF-I nor humulin significantly altered the diabetic state as measured by plasma glu-

cose levels or glycated hemoglobin values (Table 1), which reflect the short- and long-term glycemic states, respectively.

Neuroaxonal Dystrophy in Superior Mesenteric Ganglia

Ultrastructural examination of the SMG of the Pre-Rx and No Rx groups of diabetic rats demonstrated neuroaxonal dystrophy (Figure 1), the neuropathologic hallmark of sympathetic autonomic neuropathy, which we have described in detail previously in aged and diabetic rats.^{25,51} Swollen dystrophic axons and synapses were typically found intimately apposed to principal sympathetic neurons, often within their satellite cell sheaths (Figure 1A), or in the immediately adjacent neuropil. Neuroaxonal dystrophy in sympathetic ganglia in aged and diabetic rats consisted of swollen preterminal axons and synapses containing a variety of subcellular organelles (Figure 1A–E). Rare collections of large numbers of delicate axonal sprouts (arrows, Figure 1F) were encountered in association with and originating from dystrophic axons, suggesting a regenerative association/origin of neuroaxonal dystrophy. The ultrastructural appearance of dystrophic axons was identical in all treated and untreated diabetic and control groups, differing only in frequency. Although the contours of neuronal perikarya were distorted by large swellings (Figure 1, A and D), the appearance of the cell body was otherwise unremarkable; specifically, degenerating or chromatolytic neuronal cell bodies were not encountered.

Quantitative ultrastructural studies demonstrated a five- to sixfold increase in the frequency of neuroaxonal dystrophy in the No Rx group of diabetic rats in comparison to age-matched controls (Table 2, Figure 2). The Pre-Rx group of diabetic rats showed levels of neuroaxonal dystrophy comparable to the No Rx group. Treatment of diabetics with a 2-month course of IGF-I resulted in an 86% decrease in the frequency of neuroaxonal dystrophy compared to No Rx diabetics and an 80% decrease compared to the Pre-Rx group of diabetics (ie, the treatment starting point). The frequency of neuroaxonal dystrophy in the SMG of IGF-I-treated diabetic animals did not differ significantly from that of controls. The frequency of neuroaxonal dystrophy in humulin-treated diabetics did not differ significantly from the No Rx diabetic group.

Neuroaxonal Dystrophy in Ileal Mesenteric Nerves

Axons with the ultrastructural appearance of neuroaxonal dystrophy (Figure 3) also represent the hallmark of ex-

perimental diabetic autonomic neuropathy in ileal mesenteric nerves. Swollen axons (arrows, Figure 3A) overwhelm the tiny fascicles in which they reside and displace adjacent, otherwise normal axons (arrowheads, Figure 3A). Dystrophic axons are markedly enlarged and typically contain unusual collections of subcellular organelles (Figure 3).

The ultrastructural appearance of dystrophic axons was similar in all treated and untreated diabetic and control groups, differing only in frequency, although dystrophic axons in IGF-I and control groups were typically smaller than in Pre-Rx and No Rx diabetic and humulin-treated groups. The frequency of neuroaxonal dystrophy was expressed as a percentage of the total number of axons in each mesenteric fascicle (Table 2, Figure 4A) or as the number of dystrophic axons in each mesenteric nerve fascicle (Table 2, Figure 4B) with comparable results. The total number of axons composing each fascicle (Table 2) did not differ between any experimental groups.

Discussion

The nearly complete reversal of established diabetic autonomic neuropathy in ileal mesenteric nerves and prevertebral sympathetic ganglia strongly suggests a role for IGF-I in its pathogenesis and/or therapy and, considering the fidelity of the animal model to human disease, holds promise for eventual clinical use. Although both insulin and the insulin-like growth factors have been shown to support the development and growth of cultured neuroblastoma cells and sympathetic neurons,²⁸ the effect of IGF-I on the adult autonomic nervous system is essentially unexplored. Members of the neurotrophin family of neurotrophic substances (eg, NGF brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) are provided to neurons largely as locally secreted, target-derived substances that undergo binding to nerve terminal receptors and retrograde axonal transport to the neuronal perikarya; however, a similar mechanism for IGF-I has not been established. Although there is evidence of orthograde and retrograde axonal transport of endogenous IGF-I in rat sciatic somatic nerve,⁵² the relative contribution of target-derived *versus* hematogenously supplied IGF-I is not known, particularly for autonomic neurons, and retrograde axonal transport of exogenously administered IGF-I has not been established in sympathetic axons. IGF-I receptors are found on sympathetic neuronal cell bodies, axons, and nerve terminals,⁵³ and IGF-I itself has been demonstrated in the cytoplasm of lumbar sympathetic neurons.⁵² Circulating IGF-I has direct access to sympathetic neuronal cell bodies due to the lack of a ganglionic blood-nerve barrier. Decreased levels of mRNA transcripts for IGF-I and its receptor have been

Figure 1. The ultrastructural appearance of rat superior mesenteric ganglia in experimental diabetes. **A:** Markedly enlarged preterminal axons (arrows) compress and distort an adjacent principal sympathetic neuron. Bar = 2 μ m. **B, C:** Typically, dystrophic axons are characterized by an admixture of neurotransmitter granules and delicate anastomosing tubulovesicular elements, which may be arranged as loose aggregates or compact forms (arrows, **B**), which are seen at higher magnification in **C**. **B, C:** Bars = 500 nm. **D:** Some dystrophic axons (arrow) contain granular aggregates of normal and degenerating subcellular organelles and multivesicular bodies, in this case compressing an adjacent principal sympathetic neuron. Bar = 2 μ m. **E:** Swollen dystrophic axons may contain skeins of disorganized or axially oriented neurofilaments. Bar = 1 μ m. **F:** Rarely, clusters of small regenerative axonal sprouts (arrows) are found associated with and arising from a dystrophic axon (*). Bar = 1 μ m.

Table 2. Effect of IGF-I and Low-Dose Humulin on Neuroaxonal Dystrophy (NAD) in Ileal Mesenteric Nerves and Superior Mesenteric Ganglia

	SMG (NAD no./ neuron no.)	Ileal mes nerve (NAD no./fascicle)	Ileal mes nerve (Axon no./fascicle)	Ileal mes nerve (NAD no./axon no.) × 100
Control (<i>n</i> = 7)	0.086 ± .006	0.34 ± .10	473 ± 36	6.6 ± 2.0
Diabetic, Pre-Rx (<i>n</i> = 6)	0.389 ± .059*	5.28 ± 1.34 [†]	429 ± 17	121.8 ± 31*
Diabetic, No Rx (<i>n</i> = 10–11)	0.465 ± .03* (<i>n</i> = 10)	4.07 ± 0.94 [†]	459 ± 28	77.7 ± 15.9 [†]
Diabetic + IGF-I (<i>n</i> = 8)	0.146 ± .024 [§]	0.70 ± .17 ^{§**}	457 ± 24	14.3 ± 3.2 ^{†***}
Diabetic + Humulin (<i>n</i> = 8)	0.519 ± .058* [¶]	4.2 ± .6 [†]	447 ± 22	91.2 ± 13.2 [†]

Statistical comparison: **P* ≤ 0.0001; [†]*P* ≤ 0.001 versus control group; [‡]*P* ≤ 0.0001; [§]*P* ≤ 0.001; [¶]*P* ≤ 0.05 versus diabetic, pre-Rx group; ^{||}*P* ≤ 0.0001; ^{**}*P* ≤ 0.01 versus diabetic, no Rx group.

reported in diabetic rat SCG,⁵⁴ which may reflect an autocrine or paracrine function. Serum IGF-I levels are reportedly decreased in diabetic human subjects with sensory and autonomic neuropathic symptoms compared with either non-neuropathic diabetics or controls,³⁸ and one of its binding proteins (IGFBP-1) is significantly increased in diabetic neuropathic subjects.⁵⁵ IGF-I is well positioned, therefore, to participate in the pathogenesis as well as the therapy of sympathetic autonomic neuropathy.

The mechanism underlying an IGF-I effect, however, may be complex⁵⁶ and involve changes in numbers or function of IGF-I receptors as well as levels of multiple local or systemic binding proteins (IGFBP 1–6), or IGF-I and IGFBP proteases, all of which may inhibit or enhance IGF-I availability or, by binding in the extracellular matrix,⁵⁷ result in local intraganglionic effects. IGF-I effects on neurons may be exerted through influence on calcium channels,⁵⁸ expression of tubulin and neurofilament cytoskeletal genes,⁵⁹ or alterations in other possible inte-

grated mechanisms (eg, induction of other neurotrophic substances, polyol pathway) that may interact cooperatively in the diabetic milieu.

Experimental crush injury to the sciatic nerve of diabetic rats is followed by a delayed onset and/or diminished rate of regeneration of large myelinated axons compared to nondiabetic age-matched controls.³⁶ IGF-I and NGF, growth factors with roles in collateral axonal sprouting and/or regeneration, are increased in the distal stump of injured rat sciatic nerve; however, in diabetic rats this axotomy-induced increase in growth factors is blunted,^{30,35} which may underlie deficits in axonal sprouting or regeneration in diabetic somatic nerves. Evidence has also accumulated supporting the participation of abnormal collateral sprouting, frustrated axonal regeneration, and loss of synaptic plasticity ("synaptic dysplasia") in the pathogenesis of neuroaxonal dystrophy.⁶⁰ Significantly, IGF-I and IGF-II are thought to have a role in normal synaptic development as well as nerve terminal plasticity, collateral axonal sprouting, and axonal regeneration.^{30,60–65} Axonal regeneration and collateral axonal sprouting represent different, although related processes; eg, the process of collateral axonal sprouting, but not axonal regeneration, is particularly sensitive to NGF.⁶⁶ Exogenously administered IGF-I or IGF-II enhances the number of regenerating axons and functional recovery from sciatic nerve crush injury in experimental rat diabetes⁶⁷ and axotomized mouse sciatic nerve.⁶⁸ Administration of IGF-I results in increased levels of GAP-43 (a growth cone protein that is associated with regeneration and synaptic plasticity) mRNA in motor neurons.⁶⁹ Similarly, transgenic mice overexpressing IGF-I in skeletal muscle show enhanced peripheral nerve regeneration.⁷⁰ Interference with IGF-II function in regenerating injured rat somatic nerves by the application of antibodies against IGF-II results in significantly decreased axonal regeneration.⁷¹ A role for IGF-I in routine synaptic turnover and its deficiency in diabetes could underlie synaptic dysplasia and the development of neuroaxonal dystrophy.

Previous studies in experimental animals^{25,51} and human subjects^{40,41} have demonstrated that dystrophic axons also develop in sympathetic ganglia in the absence of diabetes as a function of aging alone. Dystrophic axons in the aging sympathetic nervous system are identical in anatomical distribution, immunohistochemistry,

Figure 2. The effect of IGF-I and low-dose humulin on the frequency of neuroaxonal dystrophy in the superior mesenteric ganglia of rats with experimental diabetes. The frequency of neuroaxonal dystrophy in rat superior mesenteric ganglion is presented as the ratio of numbers of dystrophic axons to numbers of nucleated neurons for the numbers of animals given in Table 2. Statistical comparisons are shown in Table 2.

Figure 3. The ultrastructural appearance of neuroaxonal dystrophy in rat ileal mesenteric nerves in experimental diabetes. **A:** The appearance of a small nerve fascicle is dominated by the presence of numerous dystrophic axons (**arrows**) admixed with nondystrophic unmyelinated axons (**arrowheads**). Bar = 5 μ m. **B, C:** Typically, dystrophic axons contain anastomosing tubulovesicular elements, ranging from coarse tubules (**arrow**, **B**) to patches of compacted elements (**arrows**, **C**) admixed with other subcellular organelles. **B, C:** Bars = 1 μ m. **D:** Markedly swollen dystrophic axons may contain collections of normal and degenerating subcellular organelles. Bar = 5 μ m.

Figure 4. The effect of IGF-I and low-dose humulin treatment on the frequency of neuroaxonal dystrophy in the ileal mesenteric nerves of rats with experimental diabetes. The frequency of neuroaxonal dystrophy in rat ileal mesenteric nerves is shown as a percentage of total mesenteric nerve axons (**A**) and as numbers of dystrophic axons per fascicle (**B**). Statistical comparisons are shown in Table 2.

and ultrastructure to those developing earlier and in exaggerated numbers in diabetes. The observation that levels of serum IGF-I and IGF-II also decrease with aging in human subjects⁷² suggests a possible common shared mechanism in aging and diabetes resulting in sympathetic neuroaxonal dystrophy. It has been proposed that IGF-I supports the normal peripheral nervous system and is diminished in diabetics with a superimposed additional age-related decrease in IGF-II, resulting in the apparent age-dependency of some forms of diabetic neuropathy.³⁰

Extrapolation of the results of animal studies of diabetic symmetrical sensorimotor neuropathy to therapeutic issues in clinical diabetic neuropathy has often been disappointing, perhaps reflecting the substantial differences between model systems and human diabetics, the chronicity of the process in humans, and the difficulty of study of the basic pathogenetic features in humans that may develop and progress unnoticed in early stages. In our studies of sympathetic ganglia of diabetic rats and human subjects conducted to date, we have shown substantial fidelity of the animal model to the human condition. Specifically, chronically diabetic rodents and human

diabetics 1) develop the characteristic axonal swellings of neuroaxonal dystrophy involving preterminal axons and synapses in sympathetic ganglia^{25,40,41} in the absence of the loss of significant numbers of sympathetic neurons^{17,25}; 2) develop ganglionic neuroaxonal dystrophy prematurely and in greater numbers than nondiabetic subjects; 3) develop individual dystrophic axons that are immunohistochemically and/or ultrastructurally identical to those that eventually appear in aging nondiabetic sympathetic ganglia; 4) demonstrate a markedly increased frequency of neuroaxonal dystrophy in prevertebral sympathetic SMG and celiac (CG) ganglia compared to the paravertebral sympathetic SCG^{25,41}; and 5) demonstrate a predilection for the involvement of selected subpopulations of nerve terminals, typically defined by neuropeptide content, while completely sparing other adjacent subpopulations.^{41,73}

Some diabetic patients with alimentary dysfunction (ie, gastroparesis, diarrhea, constipation) also show abnormal electrophysiological function of the small intestine characterized by the occurrence of noncoordinated bursts of electrical activity.⁷⁴ Such electrical hyperactivity, which does not result in effective propulsion of alimentary contents, is thought to involve both sympathetic and parasympathetic dysfunction. Diabetics with chronic constipation may have little resting abnormality of gut function and yet frequently lack a gastrocolonic reflex.⁷⁵ Therefore, rather than resulting from a simple deficit in any single effector pathway, diabetic alimentary tract dysfunction may proceed from the inability to integrate portions of the complex pathways involving parasympathetic, sympathetic, visceral sensory, and the intrinsic nervous system of the gut. A significant amount of the integration of alimentary reflexes is accomplished in the prevertebral sympathetic ganglia (celiac, superior and inferior mesenteric) we have studied. Although the role of neuroaxonal dystrophy in prevertebral sympathetic ganglia in the pathogenesis of alimentary dysfunction in diabetic human subjects is unproven at this time, classic neuropathological studies⁷⁶ of a small series of autopsied young diabetic patients with symptomatic alimentary dysfunction (ie, gastroparesis, diabetic diarrhea, and/or chronic constipation) have demonstrated numerous dystrophic axons in prevertebral celiac sympathetic ganglia, lesions that would be unlikely to develop as a function of age alone.⁴¹

Summary

We have identified a pathological process targeting selected subpopulations of terminal axons and synapses in prevertebral sympathetic ganglia and the noradrenergic axons originating from principal sympathetic neurons in the ganglia that innervate the alimentary tract. This process is poised to produce disorganization of ganglionic function and to contribute to the loss of integrated reflexes that is characteristic of clinical diabetic autonomic neuropathy. The nearly complete reversal of unambiguous diabetes-related neuroaxonal dystrophy by a 2-month course of IGF-I encourages the detailed system-

atic investigation of the role of this multifaceted growth factor in the adult sympathetic nervous system.

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