Diabetes Mellitus and the Sensory Neuron

C. Toth, MD, FRCP, V. Brussee, PhD, C. Cheng, MD, and D. W. Zochodne, MD, FRCP

Abstract. Sensory neurons in diabetes may be primarily targeted by diabetes and their involvement may account for prominent sensory loss and pain in diabetic patients. Previous studies demonstrating evidence of excessive polyol flux, microangiopathy, and oxidative stress involving sensory axons and ganglia have been joined by more recent work demonstrating altered neuron phenotype, mitochondrial dysfunction, ion channel alterations, and abnormal growth factor signaling. As such, an interesting and unique panoply of molecular changes in primary sensory neurons has been identified in diabetic models. Insulin deficiency and subsequent changes in second messenger signaling may also play an important role in how sensory neurons respond to diabetes. Applying approaches to support sensory neurons in diabetes may be an important therapeutic direction in diabetic patients.

Key Words: Diabetes; Dorsal root ganglion; Neuron; Neuropathy.

INTRODUCTION

Human diabetes mellitus is associated with “endstage” complications involving the retina, kidney, and peripheral nerve. Although exceptional control of diabetes-related hyperglycemia may attenuate, delay, or prevent development of diabetic complications, reversal of complications does not occur. Among these complications, diabetes selectively targets the peripheral nervous system in a widespread or diffuse fashion leading to polyneuropathy or selectively causing focal neuropathies. In patients with polyneuropathy, sensory and perhaps autonomic neurons appear to be involved early. Unfortunately, polyneuropathy is also not really an “endstage complication” but may, for example, develop in diabetic children.

A number of hypotheses have been proposed to explain the targeting of peripheral nerves by diabetes (Fig. 1). We will emphasize, however, those changes that are particularly directed toward the cell body or perikaryon of the sensory neuron. In the past, the direct targeting of perikarya in ganglia has not been emphasized as much as changes in distal axons. The perikaryon is an important target to consider, however, since loss of sensory neurons may be an irretrievable consequence, limiting options for repair. Alternatively, specific and early approaches that would support the sensory neuron may provide options to prevent such loss. This might be accomplished by better understanding of the potential role of neuron growth factors, including insulin.

Why would disease of perikarya account for the features of diabetic polyneuropathy? The innervation of the most distal target organs is the first to be disrupted by early diabetic polyneuropathy. This causes clinical symptoms and signs of polyneuropathy, first in the toes and then in the fingers. There may be at least 2 explanations for why longer axons and their terminals are involved. It may be that a longer “exposed” length of axon has a higher likelihood of being targeted by blood-born or systemic damage. Another reason may be that impaired perikarya lose their ability to supply the molecules or other structural components to their most distal terminals. Such terminals consequently atrophy and then disappear. Examining the epidermal innervation of the skin in patients or animals with diabetes does, in fact, identify such abnormal terminals with irregular contours and “retraction bulbs.” In older parlance, this process was termed “dying back,” although it was unclear whether the axon alone or first its cell body was damaged.

Clinical Features of Diabetic Neuropathies

Diabetic sensory polyneuropathy usually precedes involvement of motor neurons in humans. In human studies, there is loss of axons in the sural nerve as well as in the epidermis of the skin, and loss involves both myelinated and unmyelinated axons. Symptoms of sensory polyneuropathy generally begin with paresthesias, tingling, prickling, and “pins and needles”-like sensations that, with progression, travel further up the foot and leg, later involving fingers and hands. A characteristic feature of diabetic polyneuropathy is a stocking and glove distribution of pain, paresthesiae, or loss of sensation. Like other polyneuropathies, these features indicate susceptibility, as discussed above, of the longest axons (i.e. those involving the toes) to be lost first. Pain descriptors often used include burning, electrical-like sensations, aching, tightening, and discomfort by light touch (tactile allodynia). While prominent pain has been thought to be due to damage specifically to small myelinated and unmyelinated afferents subserving nociception, more recent studies have failed to support this “small fiber painful neuropathy” phenotype. Sural nerve biopsy studies of patients with diabetic painful neuropathy have identified loss of large myelinated axons as well (1). It may be that more
relevant processes in pain development are the alterations in ion channel distribution and properties with associated impacts on nerve excitability, rather than simple loss of a fiber class. Such changes are described below.

Frank sensory loss develops to pinprick perception, temperature sensibility, light touch, vibration perception, and proprioception. Such loss of protective sensation is associated with the development of foot ulceration, joint damage (Charcot joint), and sometimes amputation. Semmes-Weinstein monofilaments that bend when applied to the foot at a defined force are sometimes used to screen for such sensory loss. Distal to proximal progression of sensory loss in the foot may be associated with Achilles tendon areflexia. Loss of proprioception occurs when afferent loss has progressed more rostral with interruption of more proximal tendon and muscle afferents sensitive to distal joint movements. Similarly, marked gait unsteadiness develops later. The extent of the sensory deficit can be more accurately measured using quantitative sensory testing, which provides threshold values for vibration sensitivity, cold perception, and heat sensation. Quantitative sensory testing has identified loss of vibration sensitivity threshold as a very sensitive early index of diabetic sensory polyneuropathy.

Small (3 mm) punch biopsies of the skin can be used to quantitatively measure numbers of epidermal and dermal axons labeled with the marker PGP 9.5. In diabetes, both a loss of epidermal axons and altered appearances with formation of ovoids occur. Sensory nerve conduction studies are the most widely used and validated tests of axon integrity in diabetic patients. In diabetes, declines in the amplitude of the sensory nerve action potential occur first, followed by slowing of sensory conduction velocity. Although sural nerve biopsies are not taken for routine diagnostic purposes in diabetic patients, they identify diffuse or multifocal loss of axons, axonal degeneration, regenerating axon clusters in a single basement membrane (“pseudo-onion bulbs”), segmental demyelination, and changes in microvessels, including microthrombosis, basement membrane thickening, endothelial cell reduplication, and smooth muscle proliferation (2, 3). Some features of microangiopathy may occur in patients with early neuropathy, but whether these changes occur in parallel with axon loss or induce this loss is debated (1). Theriault et al (4) directly measured nerve blood flow using laser Doppler flowmetry in diabetic patients with early polyneuropathy who later underwent sural nerve biopsies. Despite declines in sensory nerve...
action potential amplitudes and numbers of axons (correlated with one another), local blood flow measures tended toward higher, rather than lower, values in diabetic patients, particularly those with more significant neuropathy.

While axonal atrophy contributes to conduction velocity slowing, such atrophy develops much later, at least in experimental models, and atrophy in human diabetic sural nerve biopsies has been difficult to demonstrate. Segmental demyelination, the hallmark of acquired demyelinating peripheral neuropathies, also occurs in diabetes and can contribute to conduction velocity slowing, but is generally not observed in experimental models where slowing is present. Finally, in the BB Wistar model of Type I diabetes, one group of investigators has suggested that paranodal abnormalities known as axoglial dysjunction are important structural changes that may lead to conduction slowing (5).

None of the therapies proposed to date truly reverse diabetic sensory polyneuropathy, including trials of adalose reductase inhibitors, recombinant human nerve growth factor, and many others not reviewed here. Even pancreatic transplantation, rendering euglycemia has only generated marginal improvements in neuropathy, particularly autonomic function, when patients are followed for several years.

Potential Mechanisms of Diabetic Polyneuropathy

Convergent thinking is beginning to link established derangements in diabetes into an overall scheme of the pathogenesis of its complications, including neuropathy. Briefly, these include excessive polyol (sugar alcohol) flux into nerve and probably ganglia, oxidative stress, glycosylation of nerve proteins, accelerated functional and structural microangiopathy, and failure of neurotrophic support. Schwann cells accumulate excessive polyols (6), particularly sorbitol, by accelerated flux through the aldose reductase pathway. There are associated depletions of nerve myo-inositol, changes in protein kinase C (PKC) subunits, and dysfunction of nerve Na/K ATPase (7–9). Increased axonal Na content and later Na channel migration out of nodes of Ranvier may account for early nerve conduction velocity slowing in diabetes (10, 11).

Oxidative stress is an early and critical pathogenic event (12). Auto-oxidation of glucose and glycation products of glucose generate oxygen free radicals that include the hydroxyl radical (OH•), superoxide anion (O2•−), hydrogen peroxide, singlet oxygen, nitric oxide (NO), and organic analogues. Moreover, generation of these species occurs in the setting of lowered antioxidant defenses characterized by lowered superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. The consequences of oxidative stress include lipid peroxidation (elevated malondialdehyde, and conjugated dienes), DNA damage (8-hydroxy-2′-deoxyguanosine), and protein damage (protein carbonyls). Oxidative stress may primarily target microvessels, in turn leading to vascular damage and tissue ischemia. In peripheral nerve there is an increase in malondialdehyde, conjugated dienes, and lipid hydroperoxides with reduced levels of GSH (reduced glutathione) and glutathione peroxidase (12). Hyperglycemia and oxidative stress generate advanced glycosylation endproducts (AGEs) that are thought to bind to receptors for AGEs (RAGEs), activating NF-κB, a potential mediator of cellular dysfunction (13).

Consumption of NO (nitric oxide) by AGEs in diabetes may contribute to loss of vasodilation (functional microangiopathy) in diabetic microvessels. Alternatively, there may be nitrergic stress, referring to damage of cellular proteins by excessive NO or its metabolites acting as free radical agents. NO combines with the superoxide radical (O2•−) to generate peroxynitrite (ONOO−), a highly potent oxidizing agent that nitrates protein tyrosines, eventually leading to cell death (14). NO itself targets thiol groups on proteins, such as SNAP 25 and B50/GAP43, and damages DNA, in turn activating PARS (polyADP-ribose synthase), an enzyme that depletes cellular ATP. Peripheral nerves and sensory ganglia in experimental diabetes may have elevated NO synthesis, a feature that may contribute to local radical stress (15).

Microangiopathy, at first functional, then structural, may arise from endothelial damage and NO quenching during oxidative stress and polyol flux (2, 16–18). These abnormalities are exacerbated by hyperviscosity, loss of erythrocyte deformability, increased platelet aggregation, and alterations in local oxygen release, all of which likely contribute towards an eventual cascade of hypoxia and ischemia (12, 19). Our lab, and others, have provided extensive argument against the concept that early nerve trunk ischemia “accounts” for diabetic neuropathy (see review [3]). Instead, microvascular, axon and neuron damage may develop in parallel.

Dorsal Root Ganglia and Diabetic Microangiopathy

Dorsal root ganglia (DRG) are invested with microvessels originating from segmental radicular arteries and anastomoses from spinal arteries. Sensory ganglia have interesting physiological features that may reflect greater potential vulnerability to diabetes (20). DRG entrain higher levels of local blood flow measuring 30 to 40 ml/100 g/min compared to 15 to 20 ml/100 g/min in the sciatic nerve trunk. Mean ambient oxygen tensions measured using polarography within ganglia are lower than those of the peripheral nerve trunk, indicating greater metabolic demand and oxygen uptake (21, 22). Peripheral nerves have a near linear relationship between mean arterial pressure and blood flow, whereas DRG have evidence of partial autoregulation. Combined, these physiological features of ganglia suggest heightened...
flow-metabolic coupling in ganglia and may predispose to more serious consequences of microangiopathy in diabetes (23, 24). DRG also have a more leaky blood-ganglia barrier than within nerve or brain.

We observed reductions in ganglia blood flow in experimental streptozotocin (STZ)-induced diabetes, with measurements around 20 ml/100g/min (25, 26). These changes were selective for ganglia, as the same cohort of diabetic rats had normal concurrent nerve blood flow. While such reductions might suggest that chronic ganglion ischemia damages diabetic sensory neurons primarily, it is also possible that such changes are secondary as a result of decreased neuron metabolic demand.

Could episodic ischemia target ganglia? Endothelin, a highly potent circulating endothelial elaborated vasoconstrictor has been reported to be elevated in some studies of diabetic patients, but not all (27, 28). Exposure of ganglia to exogenous endothelin does induce local vasoconstriction. Like the nerve trunk, however, its actions on diabetic microvessels are more intense and prolonged than those on similar nondiabetics (29). This finding supports the concept that microangiopathy occurs early in diabetes even if it is not a primary pathogenetic event. Endothelin vasoconstriction-induced ischemic damage and DNA fragmentation was largely selective to diabetic ganglia damaging perikarya (29). There was also downstream axonal degeneration of sensory axons in the sural nerve not directly exposed to ischemia. Intraganglionic axonal damage was observed early with axotomy cell body reactions, including eccentric nuclei and central chromatolysis.

Diabetic Sensory Neurons: Survival and Phenotype

In human diabetic subjects, biopsies of the sural nerve identify loss of axons, segmental demyelination, and structural changes in microvessels. While axon loss might arise from retraction of the axon terminals despite preservation of ganglion neurons, it might also arise from irretrievable loss of the entire neuron, including perikaryon and axon. Surprisingly, few pathological studies of human sensory ganglia in diabetes mellitus have been published. Greenbaum et al described loss of neurons, replacement with nests of Nageotte, axon retraction bulbs, and vacuolation of sensory neurons in 3 patients with clinical diabetic polyneuropathy (30). In retrospect, vacuolar changes in ganglia sensory neurons, including subplasmalemmal scalloping, may not represent genuine disease, but instead an artifact of tissue handling. Some vacuolar changes observed in neurons represent swollen double membrane mitochondria; their prevalence directly related to postmortem interval (31) (Fig. 2). As such, reports of microvacuolar neuronopathy in experimental diabetes may similarly not reflect disease (32). Schmidt et al, however, have identified a unique dystrophic structural change in proximal axonal segments of autonomic
ganglia of long-term diabetic rats (8- to 12-month duration) and in human sensory ganglia from diabetic subjects or older persons (Fig. 3) (33). Dystrophic proximal axons appear strictly localized to ganglia, sometimes indenting perikarya and containing prominent accumulations of neurofilament. The significance of dystrophic axons is uncertain, possibly related to arrested axoplasmic transport or a unique vulnerability of proximal axonal segments within ganglia. In separate work, similar proximal axon segments in a long-term diabetic model had prominent expression of activated caspase-3 and PARP (poly [ADP-ribose] polymerase) (34). In long-term models, lipofuscin pigment granules are more prominent in diabetic neurons (32, 35). Beyond these changes, and overall perikaryal atrophy, there appear to be few other obvious morphological distinctions between diabetic and nondiabetic neurons.

Is there actual loss of diabetic sensory neurons in experimental models? This appears to depend on the model chosen. The absence of overt loss on direct neuronal counts, for example, in the STZ rat model of Type I diabetes probably indicates that it is a milder model of human disease. Some studies have reported evidence of DNA fragmentation and presumed apoptosis in large proportions of ganglion neurons in early STZ rat diabetes (36, 37). Such findings of severe neuronal loss, however, are not matched by direct counts either in the ganglia or downstream sural nerve. It may be that some DNA fragmentation identified by TUNEL labeling or caspase activation provides evidence that sensory neurons suffer “apoptotic stress,” but nonetheless survive. We studied a long-term (12 month) model of experimental rat STZ-induced diabetes using a rigorous 3-dimensional physical dissector counting approach. Overall numbers were

Fig. 3. Proximal axonal dystrophic changes (arrows) in human DRG observed in humans with advanced age or diabetes. Loss of a neuron replaced by a nest of Nageotte (A, arrow) and proximal axon dystrophic swellings from sensory neurons (arrows, B, C are H & E stain, D–F are silver stain). Reproduced with permission (78).
preserved, corresponding with intact downstream sural axon populations (35). Both perikarya and axons, however, undergo atrophy with an apparent shift of sizes to smaller size categories. Overall, rats appear relatively resistant to diabetic neuropathy, while yet exhibiting measurable changes such as reductions in conduction velocity, axonal atrophy, and a series of molecular alterations (see below). The importance, then, of this model may be in its reflection of early changes of neuropathy preceding degeneration and loss.

In mice rendered diabetic with STZ, the situation appears somewhat different and this model may better parallel human disease. While 12 months of diabetes in a rat represents a substantial proportion of its lifespan, 6 months of diabetes in mice may provide relatively similar diabetic exposure. Both may better represent decades of human disease than the short-term models extensively reported in the literature. By 4 months of diabetes, STZ mice have evidence of active sensory axonal degeneration. By 8 months of diabetes there was evidence of loss of epidermal innervation of the hindpaw footpads but also substantial (~30%) loss of sensory neurons counted using the physical dissector approach (Kennedy and Zochodne, unpublished data). Rigorous counting of sensory neurons using dissector approaches have not been carried out in Type II models or in the BB Wistar rat model of Type I diabetes. Loss of perikarya requires that remaining intact neurons collaterally reinnervate denervated target organs. At this time, it is uncertain what barriers diabetes imposes upon collateral sprouting of a residual population of sensory neurons.

What happens to sensory neurons prior to dropout? In long-term model of STZ rat diabetes, axonal atrophy develops by 2 to 3 months, preceding later perikaryal atrophy (Fig. 4). Hyperosmolar shrinkage from hyperglycemia probably does not account for such changes...
because of the long delays in the appearance of atrophy after hyperglycemia appears. Progressive dysfunction of neurons implies a failure to elaborate critical structural nerve proteins, of which neurofilament and tubulin are key members. Both perikaryal and axonal atrophy can be directly related to a loss of neurofilaments. Distal diabetic axons appear to suffer a double hit, with failed synthesis of neurofilament and slowed axonal transport (38).

**Unique Molecular Changes in Diabetic Neurons**

While axonal and neuronal losses are absolute measurable endpoints in the progression of a progressive neurological disorder, sublethal neuronal dysfunction may be more difficult to identify. For example, declines in conduction velocity reflect an alteration in axon excitability but do not imply potential demise. While the mouse model does illustrate that neuron loss is an eventual consequence of diabetes, it may be that there is chronic adaptation of neurons to limit apoptosis. Pro-apoptotic signals generated from oxidative stress, chronic ischemia, polyol flux, and loss of trophic support may be accompanied by rises in neuronal survival signals. The overall balance and signaling in such pathways and their relation to disease duration are largely unknown. What is apparent is that chronically impaired diabetic neurons develop some molecular changes known to occur after injury. These changes are critical for neuron survival and regeneration and change of the primary phenotype from transmission to regeneration. Tomlinson, Fernyhough and colleagues pointed out the resemblance in alterations of neuron mRNAs between diabetes and injury. These alterations include a downregulation of mRNA levels, neurofilament H and L subunits (NFH, NFL), CGRP, and substance P among others. Reductions in peptide mRNAs were corrected by nerve growth factor (NGF) (39). Similarly, NGF levels in tissue targets and nerves of diabetic rats are reduced, prompting the hypothesis that NGF depletion accounts for molecular changes in diabetic sensory neurons (40).

The full repertoire of molecular changes in diabetic sensory neurons, however, differs from that of injury (Table). For example, progressive long-term diabetes was not only associated with declines in mRNA levels of neurofilament subunits (NFH, NFL), CGRP, and substance P among others. Reductions in peptide mRNAs were corrected by nerve growth factor (NGF) (39). Similarly, NGF levels in tissue targets and nerves of diabetic rats are reduced, prompting the hypothesis that NGF depletion accounts for molecular changes in diabetic sensory neurons (40).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Role</th>
<th>Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurofilament H</td>
<td>Heavy subunit of neurofilament cytoskeleton</td>
<td>↓ Hyperphosphorylation</td>
<td>(49, 74)</td>
</tr>
<tr>
<td>Neurofilament M</td>
<td>Medium subunit of neurofilament cytoskeleton</td>
<td>↓ Hyperphosphorylation</td>
<td>(35, 43, 74)</td>
</tr>
<tr>
<td>Neurofilament L</td>
<td>Light subunit of neurofilament cytoskeleton</td>
<td>↓ Hyperphosphorylation</td>
<td>(49, 74)</td>
</tr>
<tr>
<td>α-tubulin</td>
<td>Microtubule constituent</td>
<td>↓</td>
<td>(35, 49, 74)</td>
</tr>
<tr>
<td>αCGRP</td>
<td>Calcitonin gene-related peptide, neuropeptide</td>
<td>↓</td>
<td>(35, 75)</td>
</tr>
<tr>
<td>βCGRP</td>
<td>Calcitonin gene-related peptide, neuropeptide</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>TrkA</td>
<td>NGF receptor</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>Trk C</td>
<td>NT-3 receptor</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>p75</td>
<td>Low affinity neurotrophin receptor</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>PACAP</td>
<td>Pituitary adenylate cyclase activating peptide</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>SP</td>
<td>Substance P, neuropeptide</td>
<td>↓</td>
<td>(35)</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Neuropeptide</td>
<td>↓</td>
<td>(76)</td>
</tr>
<tr>
<td>GAP43/B50</td>
<td>Growth associated protein</td>
<td>↓</td>
<td>(49, 74)</td>
</tr>
<tr>
<td>Na,1,8</td>
<td>Sodium channel</td>
<td>↓</td>
<td>(48, 56)</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic nucleotide messenger</td>
<td>↓</td>
<td>(48)</td>
</tr>
<tr>
<td>PKCβ-II</td>
<td>Protein kinase C isof orm</td>
<td>↓</td>
<td>(48)</td>
</tr>
<tr>
<td>NFeB</td>
<td>Second messenger</td>
<td>↓</td>
<td>(52)</td>
</tr>
<tr>
<td>CREB</td>
<td>Cyclic AMP response element binding protein</td>
<td>↓</td>
<td>(52)</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin neuropeptide</td>
<td>↔</td>
<td>(35)</td>
</tr>
<tr>
<td>Galanin</td>
<td>Neuropeptide</td>
<td>↔</td>
<td>(35)</td>
</tr>
<tr>
<td>BCI-2</td>
<td>Anti-apoptotic protein</td>
<td>↑</td>
<td>(34)</td>
</tr>
<tr>
<td>ERK</td>
<td>MAPK second messenger</td>
<td>↑</td>
<td>(42)</td>
</tr>
<tr>
<td>JNK</td>
<td>MAPK second messenger</td>
<td>↑</td>
<td>(42)</td>
</tr>
<tr>
<td>P38</td>
<td>MAPK second messenger</td>
<td>↑</td>
<td>(42)</td>
</tr>
<tr>
<td>Activated Caspase-3</td>
<td>Cysteine proteinase</td>
<td>↑</td>
<td>(34)</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly (ADP-ribose) polymerase</td>
<td>↑</td>
<td>(34)</td>
</tr>
<tr>
<td>HSP27</td>
<td>Heat shock protein</td>
<td>↑</td>
<td>(35)</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin receptor</td>
<td>↑</td>
<td>(77)</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>↑</td>
<td>(46)</td>
</tr>
<tr>
<td>Na,1,3,1,6,1,9</td>
<td>Sodium channel</td>
<td>↑</td>
<td>(56)</td>
</tr>
<tr>
<td>β3</td>
<td>Sodium channel subunit</td>
<td>↑</td>
<td>(57)</td>
</tr>
</tbody>
</table>
Fig. 5. In situ hybridization images of L5 DRG from diabetic (12-month duration) rats (right) or littermate controls (left) probed to label the medium subunit of the neurofilament triplet protein (NfM). Diabetics had a progressive decline in the neuronal expression of mRNA for NfM, illustrated as less intense labeling on the right panels. Studies completed in the Verge laboratory, University of Saskatchewan and reproduced with permission from Zochodne et al (35). Stars indicate a DRG neuron with intense staining (left) or diminished staining (right).

cones (Fig. 5). Both α-1 tubulin and GAP43/B50 are upregulated within injured neurons. Other changes in the long-term experimental diabetic model appearing in later, but not earlier, time points include mRNAs coding for important structural and transmitter proteins or peptides: αCGRP, βCGRP, substance P, Trks A, B, and C, and PACAP (35). The full panoply of progressive mRNA changes has suggested that the diabetic phenotype reflects a degenerative rather than an injury response of the neuron. The distinction is important because it may alter how we view potential rescue approaches clinically.

Nerve injury upregulates mRNA and protein levels of the constitutive neuronal nitric oxide synthase (nNOS) in sensory neurons (41). Neither short- nor long-term rat diabetes was associated with significant changes in mRNA or protein levels of nNOS, eNOS (constitutive endothelial NOS), or iNOS (inducible inflammatory NOS). Overall NOS activity, however, measured by conversion of labeled arginine to citrulline, was elevated in both the ganglia and sciatic nerves of long-term diabetic rats (15). Moreover, sensory neuron perikarya and proximal axonal segments had immunoreactivity for nitrotyrosine, a footprint of NO presence (34). Overall, these findings have suggested the possibility that chronic rises in NOS activity, generating elevated local NO levels, might create long-term nitrergic stress in diabetic ganglia.

Heightened activity of mitogen-activated protein kinases (MAPK) in sensory neurons may be a critical signaling alteration in diabetes (42). Of the MAPK types, ERK and p38 were both activated in adult rat sensory neurons in culture by oxidative stress, and were increased in sensory neurons from 8-week STZ-induced diabetic rats. JNK was activated by high glucose and appeared in 12-week STZ rat nerve (42, 43). Chronic rises in MAPK activity may be pro-apoptotic and may influence other aspects of neuronal function. One consequence may be excessive neurofilament phosphorylation, a post-translational modification of the axon latticework that determines their spacing and perhaps longevity (43). Such changes appear to be reversible, as demonstrated by the impact of neurotrophin-3 (44) or insulin (45).

Two additional features indicate profound alterations in how sensory neurons function and handle their metabolism in diabetes. Huang et al used fluorescent video imaging of lumbar DRGs to calculate cytosolic calcium dynamics (46). Both large and small neuron populations had
cytoplasmic calcium levels twice that of controls, reversible by neurotrophin-3. The same group was imaged using rhodamine 123 to calculate mitochondrial inner membrane potential. Diabetes depolarized the potential, but this was restored by low doses of insulin (47). Kim et al reported that assays from ganglia of rats with diabetes of 4 to 6 weeks had declines in PKC isoenzyme protein levels, particularly PKCβ-II, but a higher relative membrane fraction of PKCβ-II (48). cGMP levels were also reduced in diabetics. It is, however, uncertain if the changes were specific for neurons since whole ganglion assays were used.

Given a superimposed axonal injury in diabetes, is the sensory neuron capable of mounting an appropriate “injury phenotype”? This does not appear to be the case. Tomlinson et al noted, using Northern analysis, that upregulation of mRNA of GAP43/B50 and Tα-1 tubulin occurring after axotomy in diabetic rats (10 weeks) was reduced compared to nondiabetic littermates (49). Neurofilament H and L and TrkA mRNA levels declined to lower levels in diabetics than controls. Retrograde loss of sensory neurons following nerve transection, prominent in neonates, is relatively mild in adults. Unlike the relatively massive loss of neurons that develops retrogradely in neonatal ganglia after axotomy, resistance to such loss in adult rodents may be due to other sources of trophic support besides that of target tissues. In diabetic rats, retrograde sensory neuron loss is much greater than in nondiabetic littermates, suggesting impairment of trophic support (Kennedy and Zochodne, unpublished data).

Overall, the molecular phenotype of the diabetic sensory neuron does suggest a cell “under siege” from a variety of stressors, at least for a period of time. In long-term (12 month) STZ diabetic rats, we found evidence of increased activated caspase-3 expression, a feature usually considered to be pre-mortem (34) (Fig. 6). Despite the presence of caspase-3, three-dimensional counting (discussed above), light electron microscopic appraisal of nuclear morphology, and TUNEL labeling failed to identify concurrent active apoptosis and loss (34, 35). Recent
work has clarified that caspase-3 expression need not necessarily specify imminent cellular demise (50), but what prevents its full action in diabetic neurons is uncertain. An interesting related finding in this cohort of rats was the presence of heightened PARP (poly ADP-ribose polymerase) expression, an enzyme induced by DNA damage. Might PARP, hitherto considered a mediator of cell death, instead help to protect neurons? Another interesting finding was that there were rises in HSP 27 mRNA in sensory neurons, in contrast to the nearly universal downregulation of most mRNA species examined (35). HSP27 is a chaperone protein linked to neuron injury and survival (51). We did not observe alterations in BCl-2 expression, but the full repertoire of pro- and anti-apoptotic signals in a relevant model of diabetes has not been examined. Tomlinson also reported declines in the binding of NFκB and CREB transcription factors, both of importance in survival of neurons, from 12-week diabetic rat ganglia (52).

How diabetes alters ion channel expression in sensory neurons may be relevant in considering neuropathic pain, a major clinical feature of diabetic neuropathies. While changes of channel protein synthesis have a bearing on their downstream investment into axons, their insertion into perikarya membranes may have an equally important role. Wall and Devor described the origin of ectopic discharges related to pain perception from ganglia neurons (53). Behavioral evidence of pain, mostly mechanical allodynia, does develop in diabetic models (54, 55), although only relatively short duration models have been examined. Craner et al described rises of both mRNA and protein levels of the sodium channels Na, 1.3, 1.6, and 1.9 coinciding with mechanical allodynia, but downregulation of Na, 1.8 (56). Kim et al similarly reported decreased protein levels of Na, 1.8 (48). Na, 1.8 and 1.9 tetrodotoxin-resistant channels are expressed in small sensory neurons, whereas Na, 1.3 is tetrodotoxin-sensitive and Na, 1.6 is expressed by myelinated axons at nodes of Ranvier. Again, as with the molecular changes described earlier, changes in sodium channel expression differed from those expected of axon transection (axonotmy), where declines of both Na, 1.8 and 1.9 would be expected. The mRNA for the β3 auxiliary subunit of the sodium channel may be upregulated selectively in medium sized Aδ diabetic sensory neurons, whereas the β1 subunit was unchanged (57).

Hirade et al investigated the physiological consequences of 8 months of diabetes on isolated diabetic sensory neurons (58). Overall, a rise in sodium current density through tetrodotoxin-resistant channels and a leftward shift in the voltage dependence of this conductance (to more negative values) were noted. Both changes may increase the excitability of sensory neurons, but it is uncertain whether these physiological changes occur strictly due to the rise in Na, 1.9.

Neurotrophic Growth Factors and Diabetic Sensory Neurons

The potential role of the neurotrophin family (NGF, BDNF, NT-3, NT-4/5) in supporting diabetic sensory neurons has been considered for some time (40) and has been reviewed elsewhere. BDNF, a molecule that supports medium sized sensory neurons and motor neurons, has had increases in its mRNA observed in DRGs and sciatic nerves of experimental diabetic rats; this may add a protective or reparative mechanism for neurons (59). NT-3 supports large sensory neurons associated with myelinated proprioceptive afferents. Exogenous delivery has altered some features of experimental diabetes, including conduction abnormalities, neurofilament accumulation, and levels of MAPks (44, 60, 61). Target tissue levels and retrograde axonal transport of NT-3 appear to be reduced by diabetes (62).

Two non-neurotrophin family growth factors not considered in further detail here are CNTF and GDNF. CNTF synthesis by diabetic Schwann cells may be impaired (63). In response to nerve transection, GDNF mRNA expression in Schwann cells and DRG satellite cells increases (64).

Perineuronal satellite cells are an interesting population of cells closely related to Schwann cells that surround, support, and possibly protect neurons in sensory ganglia (65). Features include an intimate association with neurons, scant cytoplasm, a high surface-volume laminar structure, and a basement membrane (65). NGF and NT-3 have both been observed to be upregulated in perineuronal satellite cells after injury, implying an important local paracrine supportive role for sensory neurons (66). In addition, such cells may scavenge free radicals and diminish oxidative stress. It is not known whether diabetes targets these important cells.

Role of Insulin and Insulin-Like Growth Factor-1 in Sensory Neurons

Hyperglycemia has long been considered the critical insult to peripheral nerves that accounts for neuronal damage. However, a loss of insulin signaling in Type I diabetics or resistance to its action in Type II diabetes might yet be relevant in the development of nerve disease. Insulin and closely related and regulated insulin-like growth factors belong to a family of growth factors mediating metabolism, growth, cell differentiation, and survival of most tissues in mammals. In the peripheral nervous system, insulin and insulin-like growth factor-1 (IGF-1) are neurotrophic factors important for neuronal survival and they influence phenotypic expression in DRG neurons. Insulin and IGF-1 stimulate survival, neuritic outgrowth, and other cellular responses within the adult sensory neuron (67). At low physiological insulin
concentrations, insulin appears to stimulate neuritic outgrowth using insulin receptors, while at supraphysiological doses, insulin binds to type I IGF receptors (68). Insulin binds to the α subunit of insulin receptors (IRs) where tyrosine autophosphorylation of the β subunit is promoted, subsequently leading to activation of the catalytic domain and phosphorylation of cellular substrates, including the insulin receptor substrate (IRS) proteins and Shc (69). The phosphorylation of IRS-1 or IRS-2 (also called 4PS1 [70]), on multiple tyrosine residues creates an active signaling complex via recruitment of various proteins, including phosphatidylinositol 3 (PI3) kinase, Grb2, Nck, Crk, Fyn, and SHP2. Insulin activity thus provides signal transduction through PI3K activation as well as Grb2/Sos association with IRS-2/4PS1 (70). The Ca2+-activated phospholipid-dependent PKC is stimulated by insulin and IGF-1.

IGF-1 is a polypeptide growth factor that is produced and released from target tissues. IGF receptors are present on neurons and assist in internalization and retrograde transport of IGF (71). Binding of IGFs to α subunits of IRs or type 1 IGF receptors occurs extracellularly and activates the kinase within the β subunit situated intracellularly. The major substrates for the IGF-1 receptor are Shc and IRS proteins. IGF-1 induces a sustained tyrosine phosphorylation of Shc and also stimulates association with Grb2 (72). Tyrosine phosphorylation of Shc occurs in parallel with IGF-1-stimulated activation of extracellular signal-regulated kinase (ERK) (72). Alternatively, IGF-1 induces a transient tyrosine phosphorylation of IRS-2 and stimulates an association of IRS-2 with Grb2. As well, immunolocalization studies show IRS-2 and Grb2, but not Shc, to be concentrated at the tip of the extending growth cone where membrane ruffling is most active. This suggests that association of Shc with Grb2 is essential for IGF-1-mediated neurite outgrowth, while the IRS-2-Grb2-PI3K complex may regulate growth cone extension and membrane ruffling (72).

IGF-1 has been demonstrated to have anti-apoptotic effects and anti-oxidant effects upon neuronal cells. Apoptotic effects of excessive glucose in cultured DRG neurons can be controlled with supplementation of IGF-1, which does not occur with supplementation of NGF (37). It is postulated that IGF-1 mediates its anti-apoptotic effect through the PI3K pathway, although this pathway must have differences from pathways stimulated by NGF, as these also act through the PI3K pathway (37). IGF-1 also upregulates uncoupling protein 3 (UCP3), a member of the mitochondrial transporter superfamily found within the inner membrane of mitochondria (73). UCP3 appears to be important in the regulation of production of reactive oxygen species in mitochondria, giving it a role as an anti-oxidant in diabetes.

Within the DRG in experimental diabetic neuropathy, changes occur in cellular expression of IGF-1; a preferential expression of IGF-1 and IGF receptors occurs in small (<25 μm) diameter DRG diabetic neurons (71). Loss of IGF neurotrophic support to DRG neurons may possibly relate to perturbed regulation of sodium channel expression and neuropathic pain observed in experimental models (56, 71).

CONCLUSION

Knowledge of the complexity of changes within the diabetic DRG and their role in diabetic neuropathy is ever increasing. Along with theories such as excessive polyol flux, oxidative stress, protein glycosylation, and accelerated microangiopathy, there are additional pathophysiological explanations such as failed neurotrophic support, downregulation and alterations of mRNA, and changes in signaling pathways involving MAPK. The DRG may be the most important site for continuing investigations into the etiologies of diabetic neuropathy and is likely the most important location for examining the prevention of neuropathic disease in diabetics. It is probable that a convergence of separate pathophysiological mechanisms leads to DRG dysfunction in diabetic neuropathy. Future research into the importance of the DRG in diabetic neuropathy may need to examine the importance of neuro-glial interactions and their role in the support of the DRG neuron.

ACKNOWLEDGMENTS

CT is a Clinical Fellow of the Alberta Heritage Foundation for Medical Research (AHFMR). DZ is a Senior Medical Scholar of AHFMR. Brenda Boake provided editing assistance for the manuscript.

REFERENCES


J Neuropathol Exp Neurol, Vol 63, June, 2004


