Commentary

Comparative Neuropathology and Diabetic Autonomic Neuropathy

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In their paper in this issue of *The American Journal of Pathology*, Schmidt and co-workers¹ extend their comparative studies of neuropathological changes of autonomic ganglia in diabetes mellitus to several mouse models of this debilitating disease. Neurological complications of diabetes are frequent, affecting both sensorimotor and autonomic components of the peripheral nervous system. Diabetic sensorimotor and autonomic neuropathy have a considerable impact on a patient's quality of life and also represent a significant economic burden for public health systems.^{2,3} Diabetic autonomic neuropathy may involve any organ innervated by the autonomic nervous system and result in significant and often concurrent cardiovascular, gastrointestinal, genitourinary, neurovascular, and metabolic dysfunction.⁴ Considerable morbidity and mortality are associated with the late stages of diabetic autonomic neuropathy.⁵ However, despite its clinical importance and a wealth of information relating to autonomic function in humans with diabetes, the neuropathology of diabetic autonomic neuropathy has, until recently, received less attention than diabetic sensorimotor neuropathy.

Neuropathology of Diabetic Autonomic Neuropathy

Schmidt and colleagues have extensively documented neuropathological alterations of autonomic ganglia in diabetes in both human autopsy material and in experimental models of diabetes. In humans and rodents, structural alterations are focused on prevertebral sympathetic ganglia that integrate input from neurons originating in the intermediolateral nuclei of the spinal cord, dorsal root ganglia, parasympathetic ganglia, sympathetic neurons, and retrograde projections from enteric neurons.⁶ In humans, nerve terminal damage or degeneration within prevertebral ganglia and autonomic nerve terminals within various end organs without significant neuron loss characterize the neuropathology of diabetic autonomic neuropathy.⁷⁻⁹ The focus of Schmidt and colleagues' present

study is the reproducible dystrophic swellings of neurites containing accumulations of neurofilaments, tubulovesicular elements and/or mitochondria that are the hallmark lesion of diabetic autonomic neuropathy. Neuritic dystrophy is much more frequent in the prevertebral celiac and superior mesenteric ganglia than in the paravertebral superior cervical ganglia. Loss of autonomic neurons distributed within somatic nerves that innervate the vasa nervorum and other vessels and sweat glands in skin may also occur.⁶

Unlike many animal models of diabetic sensorimotor neuropathy, neuropathological alterations of autonomic ganglia that are similar to changes described in humans have been observed in prevertebral sympathetic ganglia of streptozotocin (STZ) and BB/W diabetic rats as well as genetically diabetic Chinese hamsters.⁶ As in humans, loss of sympathetic neurons is not a feature of experimental autonomic neuropathy even after long durations of severe diabetes. In these rodent models, upwards of 6 months of experimental diabetes is required to develop significant evidence of neuritic dystrophy. These dystrophic swellings are amenable to various therapeutic manipulations that have been used to treat neuropathic complications of diabetes, including pancreatic transplantation, administration of neurotrophic amounts of insulin and IGF-1, or aldose reductase inhibitors, but not sorbitol dehydrogenase inhibitors, or the neurotrophins, NGF and NT-3. Interestingly, as in non-diabetic humans,⁷ neuritic dystrophy of comparable structure and distribution also appears in aged non-diabetic rodents, although it takes longer to develop and is less frequent.

Utility of Comparative Neuropathology

The recent and present work of Schmidt et al extends the yield of their comparative neuropathological study of diabetic autonomic neuropathy. Careful, systematic, and quantitative documentation of diabetes-induced structural alterations in sympathetic ganglia of humans and

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different rodent strains or species has provided unequivocal validation of the widespread occurrence of neuritic dystrophy, its relationship to the diabetic state, and a framework for investigating the pathogenesis of diabetic autonomic neuropathy. For example, the occurrence of neuritic dystrophy in prevertebral sympathetic ganglia in animal models of type 1 diabetes, such as STZ and BB/W diabetic rats that develop hyperglycemia in the context of deficiencies in circulating insulin and IGF-1, but not in Zucker diabetic fatty (ZDF) rats, an animal model of type 2 diabetes that develops comparable hyperglycemia in the presence of hyperinsulinemia, provide evidence suggesting that it is the loss of insulin- and IGF-1-mediated trophic support, and not hyperglycemia *per se* that causes dystrophic changes in these ganglia.¹⁰ Similarly in the paper in this issue of *The American Journal of Pathology*, the presence of neuritic dystrophy in non-obese diabetic (NOD) mice and STZ-injected NOD-SCID mice, which lack T and B cells but are otherwise genetically identical to their NOD counterparts, but not in non-injected NOD-SCID mice, argues against an exclusive autoimmune pathogenesis for autonomic neuropathy in these animals.¹ Clearly, clues that help define the pathogenesis of diabetic autonomic neuropathy have been an important outcome of a comparative approach using cleverly selected animal models.

Another significant outcome of Schmidt and colleagues' comparative approach is the extension of their studies to mouse models of experimental diabetes. The manipulation of established genetic models of diabetes (eg, NOD and db/db mice), utilization of selected mutations that have been bred to specific genetic backgrounds (eg, NOD-SCID mice), and clever use of and/or combination of genetic and chemical models of experimental diabetes (eg, STZ diabetic mice and STZ-injected NOD-SCID mice) have considerable ramifications for future studies of diabetic autonomic neuropathy. Neuropathological alterations appear much earlier in NOD mice (after 3 to 5 weeks of diabetes) than in rat models (after 6 months or more of diabetes). The inability to completely synchronize the onset of diabetes in NOD mice is obviated by STZ injections of NOD-SCID mice, which also has the added benefit of increasing the frequency of neuritic dystrophy in the prevertebral celiac and superior mesenteric ganglia over that observed in NOD mice. The larger number of dystrophic neurites associated with individual neurons in STZ-injected NOD-SCID mice should expedite electrophysiological analysis of the functional impact of this structural abnormality. Further, a synchronized onset of diabetes and compressed timeframe for lesion development will facilitate the design and implementation of future studies testing the utility and efficacy of potential therapeutics.

The extension of the studies of Schmidt and colleagues to mouse models of experimental diabetes has another important consequence. The existence of mice with relevant spontaneous mutations or the ability to create mice with targeted mutations of specific enzymes, neurotrophic factors, receptors, and biochemical pathways, coupled with the ability to selectively breed these defects to a particular genetic background and/or induce

diabetes with STZ may help further our understanding of the pathogenesis of diabetic autonomic neuropathy. While this approach has just begun to be applied to the study of diabetic autonomic neuropathy with the use of STZ-injected NOD-SCID mice, it has already been used to investigate the pathogenesis of diabetic sensorimotor neuropathy with some success. The relevance of polyol pathway enzymes and the influence of polyol accumulation on diabetes-induced nerve defects has been examined in STZ-injected mice with a targeted mutation of sorbitol dehydrogenase¹¹ and in STZ-injected transgenic mice over expressing aldose reductase.¹² Although these two studies offer conflicting interpretations about whether polyol accumulation has an impact on nerve conduction deficits in these diabetic mice, they illustrate the utility of this approach and the promise it holds for investigations of diabetic autonomic neuropathy.

Some Interesting Insights and Questions

Neurological complications of diabetes have long been linked to consequences of hyperglycemia, including increased polyol pathway flux and accumulation, exaggerated oxidative stress, and formation of advanced glycation end-products. As noted above, marked neuritic dystrophy is present in the insulin- and IGF-1-deficient STZ diabetic rat but not the hyperinsulinemic ZDF rats, despite comparable levels of hyperglycemia,¹⁰ and administration of exogenous of neurotrophic amounts of IGF-1 to STZ diabetic rats reversed established neuritic dystrophy in the prevertebral celiac and superior mesenteric ganglia without an effect on blood glucose levels.¹³ Furthermore, in Schmidt and co-workers' present paper, type 2 db/db diabetic mice fail to develop these structural abnormalities despite marked hyperglycemia. Similarly in diabetic sensorimotor neuropathy, insulin deficiency but not hyperglycemia has been suggested to be of greater significance in the regulation of neurotrophic and neurocytoskeletal protein synthesis suggested to underlie differences in nerve fiber regeneration in the insulin-deficient type 1 BB/W diabetic rats and the hyperinsulinemic type 2 BB/Z diabetic rats.¹⁴ While these studies highlight a fundamental difference between animal models of type 1 and type 2 diabetes with respect to insulin- and IGF-1-mediated neurotrophic support, they also illustrate another level of complexity in the multifactorial pathogenesis of this disease that is not a direct result of hyperglycemia and its consequences.

Overproduction of reactive oxygen species, in particular superoxide anion, by mitochondrial electron transport chains has been suggested to underlie glucose-induced increases in polyol pathway activity, formation of glucose-derived glycation end-products and activation of protein kinase C isoforms, thus implicating this reactive oxygen species in the pathogenesis of diabetic nerve injury.¹⁵ Although the lack of supporting *in vivo* studies has led some to contest whether the damage resulting from these three pathways is secondary to mitochondrial-derived superoxide overproduction, the detrimental effects of oxidative stress are well established (for review

see ¹⁶). In this light as Schmidt et al suggest, accumulations of mitochondria in subpopulations of dystrophic neurites in prevertebral ganglia of NOD and STZ diabetic mice may well be "hot spots" of oxidative stress. The functional state of the normal-appearing mitochondria in these accumulations is unclear, as is the relationship of mitochondrial dystrophy to abnormalities of axonal transport, neuritic degeneration, and the integrative function of these prevertebral ganglia. Alternatively, it is worth noting that mitochondrial dysfunction in sensory neurons of STZ diabetic rats is characterized by depolarization of the inner membrane, which is normalized by neurotrophic effects of insulin in the presence of sustained hyperglycemia.¹⁷

One intriguing aspect of the neuropathology of human and experimental diabetic autonomic neuropathy is that the paravertebral superior cervical ganglion is spared with respect to the occurrence of neuritic dystrophy. The basis for a difference in lesion development between paravertebral and prevertebral sympathetic ganglia is not clear, although several other distinctions parallel the difference in occurrence of neuritic dystrophy. Paravertebral and prevertebral sympathetic ganglia from STZ diabetic rats have comparable levels of the polyol pathway products, sorbitol and fructose, which respond appropriately to aldose reductase and sorbitol dehydrogenase inhibition. However, unlike prevertebral superior mesenteric ganglia that develop an accelerated appearance and exaggerated frequency of neuritic dystrophy in response to sorbitol dehydrogenase inhibition, paravertebral superior cervical ganglia are unresponsive to this manipulation.¹⁸ Immunolocalization of aldose reductase and sorbitol dehydrogenase in these ganglia might shed light on the different response to lesion development if there is also a difference in the cellular distribution of these enzymes. Another distinction concerns retrograde transport to these ganglia, where defects in the retrograde transport of radiolabeled NGF are evident in the superior mesenteric ganglion but not the superior cervical ganglion.¹⁹ However, the significance of this transport defect in relation to neuritic dystrophy is uncertain, given that exogenously administered NGF had no effect on the development of these lesions in experimental diabetes and actually increased them in control animals.²⁰

Clinical and experimental studies have yet to provide a complete understanding of the pathogenesis of diabetic autonomic neuropathy. However, these studies have revealed that a common theme in subpopulations of sympathetic ganglia and end organs innervated by postganglionic autonomic nerves is the dystrophic and degenerative alteration of distal axons and nerve terminals. The occurrence of similar changes across different species and various strains of species with and without specific mutations suggest that neuritic dystrophy and degeneration are an important component of diabetic autonomic nerve injury. Whether these lesions disrupt the integrative function of prevertebral ganglia and are part of the pathogenic process or an epiphenomena is unclear, although a detailed electrophysiological assess-

ment might resolve this issue. Such an analysis would likely be facilitated by the larger number of dystrophic neurites associated with individual neurons in STZ-injected NOD-SCID mice, but complicated by concurrent injury to enteric nerves.

In closing, Schmidt and colleagues have consistently used a systematic and quantitative comparative approach to define neuropathological alterations of prevertebral sympathetic ganglia in human and experimental diabetes. The extension of this strategy to the mouse, which is amenable to spontaneous and targeted mutation of genes relevant to the pathogenesis of diabetic nerve injury, holds the promise of enhancing our understanding of and ability to treat diabetic autonomic neuropathy.

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