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Mini review

# Diabetes and the plasticity of sensory neurons

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ABSTRACT

Diabetes mellitus targets sensory neurons during the development of peripheral neuropathy. While polyneuropathy is often routinely considered as another 'microvascular' complication of diabetes mellitus, this concept may no longer address the complexities and unique qualities of direct neuronal involvement. The list of altered molecules and pathways in diabetic neurons continues to grow and includes those related to structure, neuronal 'stress', and protection. A role for abnormal direct neuronal insulin signaling has emerged as an important contributing factor in neurodegeneration. Finally, important molecular players that influence neuronal and axon growth, such as PTEN (phosphatase and tensin homolog deleted on chromosome 10) are considered. A better mechanistic understanding of the pathogenesis of diabetic polyneuropathy may foster targeted therapies that reverse a long history of therapeutic failures.



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In this review, I address selective aspects of sensory neurodegeneration in diabetes beginning with assumptions about a microvascular etiology, the molecular footprint of diabetic sensory neurons, the role for direct neuronal insulin signaling, and new ideas governing peripheral sensory neuron plasticity.

## 1. Ischemia, microangiopathy

Polyneuropathy continues to be widely classified as a 'microvascular' complication of diabetes mellitus, alongside nephropathy, and retinopathy. This entrenched viewpoint, difficult to dispel, is challenged by new evidence. The complex differences among differentiated endorgans of peripheral nerve, kidney, and retina support unique mechanisms of diabetic targeting. Nerve, kidney, and retina are tissues that are variably vulnerable to ischemia and hypoxia. Others with comparable or greater vulnerability are only involved later in the disease, and to a lesser extent. Evidence from human or animal studies in diabetes to support an exclusive microvascular cause of polyneuropathy is not uniform. Polyneuropathy can develop in children and after relatively short durations of diabetes, clinical features that argue against a chronic form of generalized ischemia. Although studies are limited, reductions in indicator mean transit times and hypoxia in exposed human diabetic peripheral nerves are described. However,these studies were carried out in patients with longstanding disease [1,2]. Theriault et al. [3] did not identify declines in blood flow of the human sural nerve of diabetic subjects, whereas patients with more severe axon loss tended to have higher rates of flow; not 'shunt', since the approach specifically emphasized endoneurial measures. In animal models, only some laboratories have linked declines in nerve blood flow with experimental neuropathy. Several technical factors including the type of measurement, near nerve temperature control, duration of diabetes may have contributed to these discrepant

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findings, summarized previously [4]. In our own laboratory, work over two decades failed to confirm any relationship between nerve blood flow and experimental polyneuropathy in experiments carried out using a variety of models, experimentalists, and blood flow measurement approaches. A number of investigations linking improvement in experimental neuropathy with interventions that increase nerve blood flow are also reported but most have been associations only and have had limited endpoints. Since a wide range of approaches all reporting this form of success has been described, the linkage has raised skepticism. Along these lines, as far back has 1992, PK Thomas entitled a review "Diabetic neuropathy: models, mechanisms, and mayhem" [5].

Microangiopathy, or alterations in the structure and function of the microvascular supply of the peripheral nerve and ganglia (vasa nervorum), likely emerge in parallel with direct neuronal changes but are likely not causative. Microangiopathy renders vasa nervorum, the vascular supply of the peripheral nerve, more sensitive to vasoconstrictors, such as endothelin or norepinephrine [6]. Moreover, diabetes dampens rises in nerve blood flow (hyperemia) following nerve trunk injury [7]. However, microvascular disease fails to explain the cascade of associated molecular changes associated with diabetic polyneuropathy [8]. For example, axotomy is associated with rises in growth molecules such as Beta III tubulin and GAP43, whereas both decline in chronic diabetes [9,10]. The changes of neuronal gene output in diabetes indicate a degenerative rather than axonal injury response, discussed next.

## 2. Alterations in the molecular phenotype

The spectrum of molecular alterations in diabetic neurons has widened and depends on the model chosen and its duration. For example, rats with a type 1 model of diabetes induced by streptozotocin (STZ) amd maintained for 12 months had declines in their mRNA content of all three subunits of the neurofilament polymer, $\beta$III tubulin, Trk receptors for neurotrophins and GAP43 [9,10]. The decline in neurofilament subunit mRNA levels, also seen in 10 month type 1 BB/Wor rats [11], correlated with loss of ultrastructurally imaged neurofilaments in axons and rises in Ckd5, *p*-GSK-3$\beta$, SAPK/JNK, and *p*42/44, kinases that phosphorylate NfH and NfM. Increased phosphorylation may influence their spacing and longevity [12,13]. The gradual and eventual loss of key structural proteins also correlates with axon atrophy, a feature of longstanding experimental diabetes. Surprisingly; however, mice that completely lack neurofilament proteins in their axons, an interesting phenotype identified by Eyer and colleagues, nonetheless develop polyneuropathy, and it may be accelerated [14]. Thus, it is unlikely that subtle changes in neurofilament account for diabetic polyneuropathy.

Neuropeptides may be altered in diabetic sensory neurons and their terminals including $\alpha$ and $\beta$CGRP, SP or PACAP [9] [15–17]. After axotomy injury in diabetic rats, VIP, galanin, and CCK failed to rise. Specific changes in ion channel and function include changes in sodium channels or their subunits, P2X2 and P2X3 receptors, TrpV channels, HCN channels, and others. Their expression and activity may relate to the development of pain, particularly in early neuropathy before there is loss of afferents.

Unlike the declines in many mRNAs of chronic diabetes, there are rises in HSP27, a pro-survival molecular chaperone protein [9]. Overexpression of human HSP27 enhanced peripheral nerve regeneration in nondiabetic mice and in diabetic mice prevented loss of footpad thermal sensation, mechanical allodynia, epidermal axon loss, and sensory conduction changes [18,19].

Diabetic neurons upregulate apotosis and 'stress' related molecules. Actual neuronal loss, reported in some controversial early and short term models, is likely not a feature [20]. For example, one year old diabetic rats have normal sensory neuron numbers as assessed by unbiased three dimensional counts [21]. Neurons did not develop TUNEL labeling, an index of apoptosis although they did express activated caspase-3. Its expression was prominent in proximal axon segments, perikaryal cytoplasm, and occasionally in nuclei. Bcl-2 expression, an anti-apoptotic molecule, and cytochrome *c* were not altered in neurons. Type 1 diabetic BB/Wor rats had upregulation of cleaved capsase-3, Bcl-xl, and Bax without neuron loss [22]. Overall these findings support the idea that diabetic sensory neurons respond to 'stress' by upregulating apoptosis related machinery, but do not necessarily succumb. While cleaved caspase-3 was increased in the cytoplasm and nuclei of diabetic DRG neurons, we now recognize that its expression does not obligate cellular 'execution' [23–25].

NFκB is a complex transcription factor considered to be a 'stress' detecting system [26–28]. NFκB is associated with both damaging and protective signals in neurons [29]. In diabetes, it may promote neurodegeneration through inappropriate upregulation [28,29] perhaps related to its impaired ability to transcribe [30].

PARP (poly(ADP-ribose) polymerase), a DNA repair molecule, was altered in rat 12 month DRG sensory neurons [21]. PARP expression spilled over from its normal nuclear localization to the cytoplasm and proximal axon segments. Proximal axon segments undergo dystrophic changes in human and experimental DPN [31,32] and colocalize with sites of mitochondrial accumulation, oxidative stress, and activated caspase-3 [33]. Treatment of diabetic mice with pharmacological PARP inhibitors and mice lacking PARP are protected from the changes of DPN but the site of protection is uncertain [34,35].

AGEs (advanced glycosylation endproducts) result from nonenzymatic reactions (glycation) of glucose [36,37] and are permanently deposited in tissues and bind to receptors, most notably among them RAGE (receptor for AGEs) [Fig. 1A]. Whereas, type 1 diabetic mice lacking RAGE appear protected from polyneuropathy [38,39] (de la Hoz et al., unpublished data), the ZDF model of type 2 diabetes in rats had rises in DRG mRNA levels of RAGE but normal protein levels [40]. RAGE-AGE signaling may also offer neuroprotection [41].

'Nitrergic stress' from excessive NO production may contribute toward neuron damage and death in diabetes, particularly as it combines with the superoxide radical ($0_2-$) to form peroxynitrite (ONOO−). Peroxynitrite nitrates protein tyrosines [42] and activates PARP. Although, nNOS (neuronal) levels rise in DRG sensory neurons after axotomy [43], NOS subtype expression was not altered in long term DRG neurons [44] but there was a low grade rise in overall NOS activity and excessive nitrotyrosine, a footprint of NO presence in neuron perikarya and proximal axonal segments. Mice lacking iNOS (inducible), but not nNOS were protected from experimental DPN [45,45]. A peroxynitrite decomposition catalyst also conferred protection [46] [Fig. 2].

In summary, the repertoire of molecular changes in sensory neurons exposed to diabetes is unique and several features indicate a response to cellular 'stress'.

## 3. Insulin signals neurons

That insulin might possess actions beyond glucose regulation has been recognized for some time. For example, in 1972 Frazier, and colleagues [47] proposed that insulin had trophic properties resembling nerve growth factor (NGF) based on structural similarities. Insulin receptors (IRs) and insulin receptor substrate (IRS1,2), scaffolds for intracellular insulin signaling are widely expressed by peripheral neurons [Fig. 1B and C]. In the PNS, insulin facilitates nerve regeneration; whether, applied as a systemic injection or administered intrathecally where it has the capacity to access