Altered Neurotrophism in Diabetic Neuropathy:
Spelunking the Caves of Peripheral Nerve

Rick T. Dobrowsky, Shefali Rouen and Cuijan Yu
Department of Pharmacology and Toxicology, University of Kansas, 5064 Malott Hall Lawrence, KS 66045
Running Title- Diabetic Neuropathy and Altered Neurotrophism

* Correspondence should be addressed to: Rick T. Dobrowsky, Department of Pharmacology and Toxicology, University of Kansas, 5064 Malott Hall, 1251 Wescoe Hall Dr., Lawrence, KS 66045, (785) 864-3531, fax-(785) 864-5219, e-mail dobrowsky@ku.edu

# of text pages- 17
# of figures- 3
# of references- 42

Abstract- 246 words
Discussion- 4,276 words

ABBREVIATIONS- Cav-1, caveolin-1; DPN, diabetic peripheral neuropathy; eNOS, endothelial nitric oxide synthase; GDNF, glial cell-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; IGF, insulin-like growth factor; MAPK, mitogen-activated protein kinase; MEK-1, MAPK kinase 1; NGF, nerve growth factor; NRG-neuregulin; PtdIns 3K, phosphatidylinositol 3 kinase; SCs, Schwann cells; STZ, streptozotocin
Diabetic peripheral neuropathy (DPN) is a frequent and potentially traumatic complication in diabetic individuals. The chronic nature of diabetes and its associated hyperglycemic episodes initiates a complex and inter-related series of metabolic and vascular insults that contribute to the polygenic etiology of DPN. One contributing factor in DPN is an altered neurotrophism that results from changes in the synthesis and expression of neurotrophins, insulin-like growth factor and various cytokine-like growth factors that can directly act upon distinct subpopulations of sensory and motor neurons. Although considerable effort has focused upon examining growth factor signaling in hyperglycemia stressed neurons, myelin-forming Schwann cells also undergo substantial degenerative changes in DPN. However, scant attention has been devoted to understanding the effect of hyperglycemia on the response of Schwann cells to growth factors critical to their function. Neuregulins are gliotrophic growth factors that signal through members of the Erb B receptor-tyrosine kinase family. The neuregulin/Erb B ligand-receptor cassette can differentially influence the response of Schwann cells throughout their development by regulating cell survival, mitogenesis and differentiation. The activity of Erb B receptors may also be affected by their interaction with caveolin-1, a protein marker of caveolae ("little caves"). However, whether neuregulin signaling may be directly or indirectly altered under conditions of hyperglycemic stress and contribute to the physiological progression of DPN is unknown. This brief review will provide a perspective on a putative role of changes in the caveolar proteome of Schwann cells in contributing to an “altered neuregulinism” in DPN.
DPN is a common complication of diabetes that develops in about 50% of the approximately 17 million Americans afflicted with either Type 1 (~1 - 1.7 million) or Type 2 diabetes. Of serious consequence, the sensory deficits and microvascular disease of DPN foreshadow an increased likelihood of foot ulcerations and gangrenous infections that are accompanied by a 15% risk of amputation due to irreparable tissue damage. This is the second leading cause of limb amputation in the US following accidental trauma and carries a considerable cost to both quality of life and health care economics (Feldman et al., 2003).

The most prevalent form of DPN develops as a distal symmetric sensorimotor neuropathy that arises due to the degeneration of small sensory fibers that mediate pain/temperature sensation and large fibers that are involved primarily in proprioception/tactile sensation. Diabetic autonomic neuropathy may also accompany distal symmetric neuropathy and can disrupt many sympathetic or parasympathetic functions, leading to gastric paresis, changes in cardiac responsiveness and genitourinary dysfunctions (Feldman et al., 2003). Diabetics often develop subclinical neuropathy that is asymptomatic but wherein nerve dysfunction is indicated by decreased peripheral nerve conduction velocity (Dyck and Dyck, 1999). The gradual progression to a clinical neuropathy may give rise to negative (painless) symptoms that include thermal hypoalgesia, loss of vibration or pain sensation and numbness. In contrast, 10–20% of patients have positive (painful) symptoms, such as burning and/or lancinating pains, pins and needles or increased touch sensation (hyperesthesia). Unfortunately, the mechanistic origin of neuropathic pain is complex and poorly understood, rendering much of the clinical management palliative.
Biochemical Events Contributing to DPN

Although DPN has proven difficult to manage clinically, results from the Diabetes Control and Complications Trial unequivocally demonstrated that tight glycemic control with intensive insulin therapy of Type 1 diabetics could dramatically slow the onset and attenuate the progression of some of the nerve conduction deficits associated with DPN. These findings strongly implicate hyperglycemia and insulin deficiency as causative events leading to nerve dysfunction (The DCCT Research Group, 1995).

Both hyperglycemia and insulin deficiency initiate a rather dynamic and interrelated series of biochemical changes that contribute to the characteristic dying back axonopathy of long myelinated fibers (Fig. 1). However, no compelling evidence has identified that this degeneration results from a single primary lesion specifically attributable to neuronal, glial or vascular damage by hyperglycemia. Similarly, no data exists that unequivocally establishes that DPN is initiated by a single biochemical event. However, attempts to order the molecular events that lead to the major metabolic alterations associated with DPN have identified oxidative stress as an initiating biochemical lesion in hyperglycemia-stressed cultured endothelial cells (Nishikawa et al., 2000). Whether this same relationship exists in neurons and glia is unclear; but a growing body of evidence does support a central role for oxidative stress in the apoptosis of peripheral neurons and glia in diabetes. A critical role of oxidative stress in the physiological progression of DPN is also underscored by the efficacy of anti-oxidant therapies such as α-lipoic acid (Vincent et al., 2004). Nevertheless, other metabolic abnormalities arising from the production of advanced glycation end products (AGEs), protein kinase C activation and increased flux of glucose through the polyol and
hexosamine pathways clearly contribute to DPN. Finally, changes in neurotrophic support of neurons and glia also contribute to the axonopathy associated with DPN. The vital role of altered neurotrophism in influencing the balance between neuronal degeneration/regeneration in DPN has been recently emphasized in a comprehensive literature assessment (Leinninger et al., 2004). Therefore, to minimize reiteration and frame the issue of altered growth factor signaling as it relates to Schwann cell function in DPN, we provide a only brief synopsis of some of the salient literature related to altered neurotrophism in neurons.

Altered Neurotrophism in DPN

Growth factors are critical mediators that direct the interplay of molecular signals operative in neuronal and glial differentiation and in re-establishing appropriate axon-glial interactions necessary for neuronal regeneration. Nerve growth factor (NGF) is a well-characterized neurotrophin that plays an important role in the adult peripheral nervous system. Studies in the last decade indicated that NGF levels were significantly reduced in the peripheral nerves of streptozotocin-induced diabetic rats but were increased in skin. Despite the increased expression of neurotrophins in the skin, decreased retrograde transport from target tissues may contribute to nerve degeneration by reducing NGF and neurotrophin-3 levels in the soma (Hellweg et al., 1994). The decreased availability of neurotrophins and altered production of other growth factors such as insulin-like growth factor 1 (IGF-1), glial-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor have led to the hypothesis that treating animals with these proteins may alleviate some of the clinical symptoms of DPN. This
strategy is also supported by electrophysiological studies that demonstrated the simultaneous presence of degenerating and regenerating nerve fibers in DPN, indicating that DPN is a highly dynamic and progressive dying-back neuropathy that does not occur in a temporally simultaneous fashion (Apfel, 1999). Concurrent with good glycemic control, growth factor therapy may be highly efficacious in minimizing initial damage or subsequent re-injury in regenerating cells early in disease progression when the state of degeneration/regeneration is more dynamic (Fig. 1). To this end, administration of NGF, IGF-1 or GDNF have all proven effective in alleviating both behavioral and biochemical measures of sensory neuropathy in diabetic rats and/or mice (Leinninger et al., 2004). These outcomes provided a compelling rationale for pursuing the clinical assessment of neurotrophic factor treatment in human DPN. GDNF is currently in clinical trials for managing some neurodegenerative diseases, and NGF therapy has traversed both Phase II and Phase III trials in treating DPN. Paradoxically, although Phase II trials of recombinant human NGF (rhNGF) in treating DPN revealed some relief of the sensory deficits of distal sensorimotor neuropathy, no significant differences emerged in a large Phase III clinical trial that quantified multiple measures of sensory deficits between patients receiving placebo versus rhNGF. Although multiple factors may have contributed to the failure of the Phase III trial (Apfel, 2002), some of the limited success in the clinical trials of NGF therapy for DPN is attributed to our poor understanding of how diabetes-induced changes in the neuronal or glial proteome may alter the predicted response to growth factor administration. For example, recent evidence indicates that doses of insulin or IGF-1 that are insufficient to improve hyperglycemia can reverse some of the biochemical (Huang et al., 2003) and
morphological (Brussee et al., 2004) changes in DPN through direct action on neurons. Since insulin resistance is a common feature in Type 2 diabetics, these data suggest that increased insulin resistance in neurons (altered neurotrophism) may also directly contribute to the development of DPN in conjunction with the overlying episodes of hyperglycemia in these patients (Brussee et al., 2004). Therefore, to aid in predicting cellular responses to growth factors and enhancing their potential clinical effectiveness in DPN, it is important to determine whether diabetes alters normal signal transduction events in neurons and glia.

As is evident from the brief discussion above, considerable attention has been given to examining sensory nerve function in diabetes in response to growth factors. However, neurons are often inter-dependent on Schwann cells (SCs), which also undergo substantial degenerative changes in DPN and respond to many of the aforementioned growth factors. For example, IGF-1 rescues SCs from hyperglycemia-induced apoptosis (Delaney et al., 1999) and regulates SC motility (Cheng et al., 2000), a critical feature in re-establishing axon-glial interactions. Neuregulins are a family of gliotrophic factors that transduce signals through Erb B receptor tyrosine kinases and are necessary for SC growth, survival and differentiation (Adlkofer and Lai, 2000). Intriguingly, recent evidence also supports that altered neuregulin signaling may also induce the de-differentiation of mature myelinated SCs and contribute to the development of peripheral neuropathies.
Neuregulins and Erb B in Peripheral Neuropathies

The neuregulin/Erb B ligand-receptor cassette can influence SC responses at multiple stages throughout their development. Erb B receptors are part of a family containing Erb B1 - 4. Erb B1 binds epidermal growth factor (EGF) and is better known as the EGF receptor while Erb B2, B3 and B4 bind to ligands called neuregulins (Pinkas-Kramarski et al., 1998). Neuregulins (NRG) are membrane-bound and soluble EGF-like ligands comprised of four subfamilies (NRG1-4) whose diversity is enhanced by alternative splicing and differential promoter usage (Adlkofer and Lai, 2000). Neuregulins bind with high affinity to either Erb B3 or Erb B4, which form a kinase-active heterodimer with Erb B2. However, Erb B3 does not possess an intrinsic tyrosine kinase activity but undergoes trans phosphorylation by Erb B2 upon heterodimerization.

Recent evidence indicates that Erb B family members are differentially expressed on the soma of small, medium and large neurons of the dorsal root ganglion (Pearson. and Carroll, 2004), but the functional role of this expression remains to be determined. In contrast, the cell biology of neuregulins in SC function is better characterized and directly relates to the regulation of cell proliferation, survival and differentiation. Erb B2 is highly expressed in SCs of neonatal nerve but decreases during myelination leading to a comparatively lower level of expression in the adult. However, levels of the high affinity Erb B3 receptor remain relatively unchanged during SC development (Grinspan et al., 1996). Importantly, Erb B2 and Erb B3 levels can change in response to injury and influence SC responsiveness to autocrine or axon-derived neuregulins (Carroll et al., 1997).

9
Neuregulin Signaling in the Control of Myelination and Demyelination

Precursor SCs are dependent upon NRG-1 and Erb B2/B3 for maturation, proliferation and survival since genetic deletion of nrg1, erbB2 or erbB3 resulted in a severe depletion of SC precursors (Garratt et al., 2000). Neuregulins also promote the survival of committed, axon-associated SCs (Syroid et al., 1996) and the early events of myelination (Garratt et al., 2000). Indeed, recent genetic studies provide strong evidence that axonally derived NRG1-Type III is critical to regulating myelin sheath thickness (Michailov et al., 2004). Increased myelination was specific for the NRG1-Type III isoform since transgenic overexpression of NRG1-Type 1 did not change myelin thickness (Michailov et al., 2004). Since DPN is associated, in part, with demyelination of sensory and motor neurons, it will be important to determine if decreased expression of NRG1-Type III in sensory axons may be associated with myelin degeneration and/or a decreased rate of remyelination in diabetes.

Distinct from the promyelinating effect of NRG1-Type III, several lines of evidence indicate that the neuregulin/Erb B2 ligand-receptor pair can also induce SC de-differentiation. Transgenic over-expression of NRG1-Type IIβ3 (also called glial growth factor) specifically in SCs led to a demyelinating hypertrophic neuropathy that resulted in hind-limb paralysis (Huijbregts et al., 2003). Similarly, glial growth factor, but not fibroblast growth factor-2, induced substantial demyelination of fully myelinated SC/sensory neuron co-cultures (Zanazzi et al., 2001). Demyelination was not dependent upon re-entry into the cell cycle, but de-differentiated SCs gradually began to reproliferate. Finally, the targeted expression of a dominant-negative Erb B4 in non-myelinating SCs induced a progressive small fiber neuropathy. Altered neuregulin
signaling induced by transgenic expression of the dominant-negative Erb B4 did not change sensitivity to mechanical stimuli but induced a thermal hypoalgesia. (Chen et al., 2003). Thermal sensitivity is mediated in large part by C-fibers and the thermal hypoalgesia correlated with a decrease in GDNF levels, which supports neuron survival in a specific subpopulation of C-fibers. Interestingly, Erb B signaling may provide a pro-survival signal in adult non-myelinating SCs since the rate of apoptosis was also increased by expression of the dominant-negative Erb B4 (Chen et al., 2003). Whether altered neuregulin signaling may contribute to minimizing the extent of SC apoptosis observed in DPN warrants examination. Collectively, these data provide compelling genetic and pharmacological support that altering the expression/activity of elements of the neuregulin/Erb B ligand-receptor pair is sufficient to alter the balance between regeneration and degeneration of both myelinating and non-myelinating adult SCs and induce the onset of peripheral neuropathies outside the context of diabetes.

**Erb B-dependent Signaling Cascades and the Regulation of SC Differentiation** - Neuregulin-induced formation of Erb B heterodimers links these receptors to downstream signaling cascades that can have both a negative and positive impact upon SC differentiation. Tyrosine phosphorylation of Erb B heterodimers couples these receptors to activation of p42/p44 mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PtdIns 3K). Although p42/p44 MAPK and PtdIns 3K are central to promoting SC survival, their effect on cell proliferation and differentiation vary depending upon the developmental stage of the SCs or the presence of axons (Maurel and Salzer, 2000). In this regard, activation of PtdIns 3K is critical to the progression from an immature SC phenotype to the promyelinating stage and the
expression of myelin protein markers (Maurel and Salzer, 2000; Ogata et al., 2004). Conversely, activation of the p42/p44 MAPK pathway may have an inhibitory effect on myelination since expression of a constitutively active MEK-1 (MAPK kinase) in SCs increased the phosphorylation of p42/p44 MAPK and was sufficient to inhibit the forskolin-induced upregulation of myelin-associated glycoprotein, a myelin marker protein (Ogata et al., 2004). Consistent with these results, tamoxifen-induced activation of raf-1 (MAPK kinase kinase) in myelinated SC/sensory neuron co-cultures also increased SC dedifferentiation and the loss of myelin proteins (Harrisingh et al., 2004). SC dedifferentiation did not seem to involve activation of PtdIns 3K since pharmacologic or molecular inhibition of this lipid kinase did not prevent NRG1-induced demyelination of myelinated SC/sensory neuron co-cultures (Harrisingh et al., 2004). Zanazzi et al (2001) have also observed that treating myelinated SC/sensory neuron co-cultures with glial growth factor induced extensive demyelination, but these authors observed a greater increase in PtdIns 3K activity than MAPK activity. Although the above discussion paints a rather complicated picture of the effect of neuregulins and downstream signaling events in regulating myelination, these data support the notion that the balance between p42/p44 MAPK and PtdIns 3K activity may have a significant effect on SC function in diabetes. Since p42/p44 MAPK is substantially activated in diabetic nerve (Purves et al., 2001), neuregulin-induced activation of the p42/p44 MAPK pathway may inhibit myelination and induce SC dedifferentiation in vivo.

Given the critical role of neuregulins in SC function, it is surprising that little effort has been directed toward examining the effect of hyperglycemia on the response of SCs to neuregulins or ascertained if diabetes alters the level of neuregulins and Erb B.
family members in peripheral nerve or dorsal root ganglia. Recent data from our lab using immunofluorescent analysis for phospho-Erb B2 in SCs found a very low level of activity in control nerve, consistent with previous results (Grinspan et al., 1996). In contrast, the intensity of phospho-Erb B2 immuno-staining was significantly enhanced (> 2-fold) in mice rendered diabetic for 8 weeks and strongly attenuated toward control levels following two weeks of insulin therapy (Rouen and Dobrowsky, unpublished observations). Although the mechanism by which Erb B2 activity may be affected in diabetes is unknown, it is clear that changes in the expression of proteins that differentially affect the activation of Erb B2 or its coupling to either MAPK or PtdIns 3K would be expected to have a marked impact on the contribution of neuregulin signaling in promoting myelination and/or demyelination. One such protein that is highly expressed in SCs and whose interaction with Erb B2 may affect its activity and coupling to downstream mediators is caveolin-1 (Cav-1).

Caveolin-1 in Schwann Cell Function and DPN

Cav-1 is well established as an integral membrane protein that is a primary protein component of caveolae, specialized plasma membrane domains enriched in cholesterol, sphingomyelin and glycosphingolipids. Cav-1 is the first member of a multigene family of distinct proteins termed caveolins-1, -2 and -3. The expression of Cav-1 and Cav-2 are intimately linked since Cav-1 knockout animals also have severely decreased expression of Cav-2 (Razani et al., 2001). Thus, Cav-1 and -2 are co-expressed in many cells while the expression of caveolin-3 is restricted primarily to muscle.
Despite extensive study, the exact function of Cav-1 and related family members in cell biology remains uncertain. This is especially the case for SCs since no direct function of Cav-1 has been identified. It is clear that Cav-1 serves a required structural role in the morphologic formation of caveolae since mice deficient in Cav-1 expression lack caveolae (Razani et al., 2001). The high level of Cav-1 expression in SCs (Mikol et al., 1999) would be consistent with recent morphological evidence that supports the presence of caveolae in myelinated SCs (Meier et al., 2004).

The formation of caveolae by Cav-1 is associated with the propensity of the protein to form oligomers and strongly bind cholesterol. Indeed, Cav-1 gene expression is responsive to changes in cellular cholesterol levels via two sterol regulatory elements in its promoter. Although Cav-1 serves as an integral membrane protein in the formation of caveolae, it can have a strong cytosolic localization in cells actively involved in sterol metabolism. This atypical localization is due to its role in transporting newly synthesized cholesterol in a vesicle-independent manner complexed with chaperones such as cyclophilins, heat shock protein 56 (Uittenbogaard et al., 1998) and annexin II (Uittenbogaard et al., 2002). Cholesterol comprises about 25% of myelin lipid and SCs derive all their cholesterol for myelination from de novo synthesis (Jurevics and Morell, 1995). Facilitating cholesterol transport would be consistent with the presence of a cytosolic pool of Cav-1 in SCs (Mikol et al., 1999) and increased Cav-1 expression during periods of active myelination in vivo (Mikol et al., 2002). Further, Cav-1 also strongly co-localizes with staining for myelin basic protein, a marker of myelinating SCs, suggesting a tight association of Cav-1 with myelination (Fig. 2A). However, it is important to note that Cav-1 is not necessarily essential for cholesterol shuttling but can
facilitate both cholesterol influx and efflux (Fielding and Fielding, 2000). Whether Cav-1 has a functional role in regulating myelination via cholesterol shuttling or otherwise awaits further analysis of the Cav-1 knock-out mice.

**Altered Cav-1 Expression in Diabetic Nerve**—Streptozotocin (STZ) is a glucose analog that induces diabetes in rats and mice by decreasing pancreatic $\beta$-cell mass. The resulting loss of insulin secretion induced by STZ provides a well-characterized animal model for Type 1 diabetes. Importantly, insulin replacement can help normalize blood glucose levels and reverse many of the pathophysiological changes that arise from prolonged hyperglycemia induced by STZ.

Since myelinated SCs can undergo substantial degeneration in diabetes, we hypothesized that Cav-1 expression may be altered by hyperglycemia. In the control nerve (Fig. 2B), Cav-1 immunoreactivity (green) is evident surrounding many axons identified by their positive staining for neurofilament (red). Following 9 weeks of STZ-induced diabetes, Cav-1 expression in SCs of rat sciatic nerve decreased dramatically but maintained an intense abaxonal staining in limited regions of the nerve. This may represent residual Cav-1 that remains co-localized with myelinated axons. On the other hand, the phenotypic change in Cav-1 localization mimics the profile reported for increased glial fibrillary acidic protein (GFAP) expression, a marker for non-myelinating SCs, following nerve transection (Cheng and Zochodne, 2002). Whether these areas of Cav-1 immunoreactivity represent dedifferentiated GFAP positive SCs in the process of re-entering myelination awaits a more definitive assessment. Importantly, the decline in Cav-1 expression was not a consequence of apoptosis since staining for the SC marker, S100$\beta$, revealed no substantial loss of SCs (Tan et al., 2003).
The association of prolonged hyperglycemia with decreased Cav-1 expression raises the issue of whether this is a direct or indirect effect of hyperglycemia. However, two weeks of insulin therapy strongly attenuated Cav-1 downregulation implicating hyperglycemia, as opposed to non-specific effects of STZ toxicity, as a primary event in decreasing Cav-1 expression (Tan et al., 2003). Alternatively, since STZ treated animals are insulin deficient, decreased Cav-1 expression may be a consequence of insulin deficiency. Arguing against this possibility, incubation of primary SCs in 20 mM to 40 mM glucose inhibited transcription from the Cav-1 promoter and decreased Cav-1 mRNA synthesis and protein expression. The ability to recapitulate the effect of hyperglycemia on Cav-1 expression in cultured primary SCs suggests that hyperglycemia, and not insulin deficiency per se, may be a sufficient primary effector in regulating Cav-1 expression in vivo.

Cav-1 is expressed in numerous cell types in peripheral nerve but it is important to note that diabetes did not decrease Cav-1 expression in endoneurial blood vessels or the perineurial membrane of sciatic nerve (Tan et al., 2003). Similarly, axotomy also strongly downregulated the expression of Cav-1 in SCs but had little effect on Cav-1 expression in blood vessels or the perineurium (Mikol et al., 2002). These observations suggest that the effect of diabetes on Cav-1 expression may have a very cell specific basis. Indeed, 12 weeks of STZ-induced diabetes increased Cav-1 expression 1.9 fold in caveolae purified from lung endothelium using cationic colloidal silica (Bucci et al., 2004). The increased expression of Cav-1 in lung endothelium correlated with an increase in morphologically definable caveolae and enhanced trans-endothelial transport (Pascariu et al., 2004). Cav-1 expression was also increased in aortic
endothelium of non-obese diabetic (NOD) mice, a genetic model for Type 1 diabetes (Bucci et al., 2004). The authors speculated that increased Cav-1 may inhibit endogenous endothelial nitric oxide synthase (eNOS) activity and contribute to the decreased release of nitric oxide in Type 1 diabetics (Bucci et al., 2004). This relationship is of potential physiological relevance since analysis of the Cav-1 knock-out mice clearly supports the contention that this protein serves as a physiological regulator of eNOS; a marked phenotype of these animals relates to vascular abnormalities and increased microvascular permeability due to hyper-activation of eNOS (Razani et al., 2001).

**Putative Roles of Altered Cav-1 Expression in Regulating Erb B2 Signaling in Diabetes** - The well-characterized inhibitory interaction of Cav-1 with eNOS provides an example of how Cav-1 may serve as a scaffolding protein that regulates signal transduction complexes within caveolae. Along this line, changes in the expression of Cav-1 may influence neuregulin signaling through its ability to bind directly to Erb B2 and inhibit the intrinsic tyrosine kinase activity of this receptor (Engelman et al., 1998). Given the critical role of Erb B2 signaling in SC biology, we have begun to examine the effect of hyperglycemia and Cav-1 downregulation on neuregulin signaling. Either hyperglycemia or forced downregulation of Cav-1 was sufficient to increase neuregulin-induced thymidine uptake in primary SCs. Since Cav-1 downregulation was associated with prolonged Erb B2 tyrosine phosphorylation (Tan et al., 2003), we hypothesized that changes in Cav-1 expression may alter downstream components of neuregulin signaling through Erb B2. Indeed, expression of a Cav-1 antisense adenovirus in SCs increased the magnitude and prolonged the activation Erb B2 and the PtdIns 3K/Akt
pathway, but not the p42/p44 MAPK pathway (Yu and Dobrowsky, unpublished observations). Recent evidence in endothelia cells also supports this hypothesis since stimulation of primary endothelial cells with vascular endothelial growth factor following Cav-1 downregulation by siRNA resulted in enhanced activation of the PtdIns 3K/Akt pathway with little effect on the MAPK pathway (Gonzalez et al., 2004). These studies provide proof-of concept that altering Cav-1 expression can differentially affect the coupling of growth factors to downstream signaling pathways.

As discussed in the previous sections, strong genetic evidence clearly supports a potential physiological role of altered neuregulin signaling in contributing to peripheral neuropathies independent of diabetes. Although results from the diabetic animal models support that prolonged hyperglycemia leads to a decrease in Cav-1 expression that correlates with an increase in Erb B2 phosphorylation in vivo, the relationship between these events in contributing to SC degeneration and DPN remains unclear. However, the available data have led us to develop a working model for the putative relationship between Cav-1 and Erb B2 in contributing to an “altered neuregulinism” in the progression of DPN (Fig. 3). Clearly, the progressive downregulation of Cav-1 in SCs is a consequence of both metabolic (Tan et al., 2003) and physical (Mikol et al., 2002) insults to peripheral nerve. In vitro data indicate that the gradual loss of Cav-1 expression may remove an inhibitory regulation of endogenous Erb B2 and contribute to an increase in its tyrosine kinase activity. Prolonged activation of Erb B2 in myelinated SCs from axon-supplied neuregulin may lead to activation of the PtdIns 3K and/or MAPK pathways. The differential ability of these pathways to decrease or increase expression of myelin proteins at specific developmental stages of the SCs may
contribute to the highly dynamic degeneration/ regeneration of myelinated axons in DPN.

Summary and Conclusions

Hyperglycemic episodes initiate a complex and inter-related series of metabolic and vascular insults that contribute to the polygenic etiology of DPN. Altered neurotrophism in both SCs and sensory neurons contributes, at least in part, to the degeneration of peripheral axons that is a clear consequence of prolonged diabetes. Although the role of both neuregulins and Cav-1 in DPN is unknown, intensive analysis of the Cav-1 knock-out animals has not identified Cav-1 as the monogenic cause of any specific disease. On the other hand, the data strongly indicate that decreases in Cav-1 may have cell specific effects on insulin receptor expression and cell signaling events relevant to both cardiovascular disease and diabetes (Cohen et al., 2003). Comparing the effect of diabetes between wild type and Cav-1 null animals on rates of myelination/demyelination, Erb B2 activation, and the onset of sensory deficits in diabetes will provide genetic insight into the potential role of this protein in affecting sensory dysfunction in DPN. Similarly, use of primary SCs from the Cav-1 null animals will prove valuable in assessing the sufficiency and necessity of this protein in regulating neuregulin signaling and affecting the expression of myelin proteins. Although caves are gloomy upon entry, spelunking into SC caveolae may shed light on enhancing the effectiveness of existing and novel growth factor therapies in the difficult therapeutic management of DPN.
Acknowledgements

We would like to thank Dr. M. Michaelis for her critical review of the manuscript.

Editorial policy limits citations to being illustrative and we apologize for excluding the many important contributions of numerous authors.
References


Footnotes

This work was supported by grants from the American Diabetes Association and the National Institutes of Health (NS38154)
Figure Legends

Fig. 1. Schematic of Biochemical and Physiological Parameters Contributing to DPN. Hyperglycemia is an initiating event leading to a series of metabolic changes that may originate from increased oxidative stress. These metabolic insults contribute to impairments in the nerve and vasculature leading to the subsequent axonopathy of DPN. Altered neurotrophism may influence the rate of change in the dynamic balance between regenerating and degenerating neurons/glia and eventual apoptotic cell loss. The gradual progression of sensory dysfunctions increases with the duration of diabetes.

Fig. 2- Cav-1 Co-localizes with Myelin Basic Protein and Decreases in SCs of Sciatic Nerve from Diabetic Rats. A) Left panel shows immuno-staining for myelin basic protein surrounding numerous unstained axons. Right panel shows the merged image of staining for Cav-1 (red) with myelin basic protein (green). Evident is the extensive overlap indicated by the intense yellow color obtained after merging the images. However, both Cav-1 and MBP show regions of minimal overlap. B) Left panel shows staining of rat sciatic nerve from a control animal for Cav-1 (green) and the axonal marker neurofilament (red). Cav-1 staining (arrows) is evident surrounding most axons throughout the section. Right panel shows staining pattern obtained for the same proteins following 9 weeks of diabetes. Evident is the extensive loss of Cav-1 throughout the nerve with some residual staining still associated with select axons.
Fig. 3- Schematic Presentation for Hypothesized Role of Cav-1 in Regulating Neuregulin Signaling in SCs. Hyperglycemia downregulates the expression of Cav-1 (red) in SCs leading to the loss of an inhibitory interaction of the Cav-1 scaffolding domain (open box) with Erb B2 tyrosine kinase domain (green). Axon-derived neuregulin may stimulate formation of Erb B2 (green) and Erb B3 (black) heterodimers leading to the activation of PtdIns 3K and p42/p44 MAPK pathways. Activated MAPK (ppMAPK) and/or PtdIns 3K may promote the dedifferentiation of myelinated SCs leading to decreased axon-glia interactions that contribute to the progression of nerve dysfunction. Blue shading indicates cysteine rich domains of Erb B receptors.
Initiating Insult

Metabolic Changes

Physiologic/Morphologic Alterations

<table>
<thead>
<tr>
<th>Oxidative Stress</th>
<th>Nerve Conduction Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperglycemia</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polyl Pathway</th>
<th>Microvascular Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexosamine Pathway</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGEs</th>
<th>Neuronal/Glial Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Neurotrophism</td>
<td>Neuronal/Glial</td>
</tr>
</tbody>
</table>

Regeneration/degeneration

Neuronal/Glial regeneration/Degeneration

Sensory Dysfunction

Time
Hyperglycemia

NRG1

MAPK

ppMAPK

Dedifferentiation

Decreased Axon/Glia Interaction

Decreased Nerve Function?

Euglycemia

p85 PI3K

p110 PI3K

p85 PI3K

p110 PI3K

?