Diabetic Peripheral Neuropathy: Should a Chaperone Accompany Our Therapeutic Approach?

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Abstract—Diabetic peripheral neuropathy (DPN) is a common complication of diabetes that is associated with axonal atrophy, demyelination, blunted regenerative potential, and loss of peripheral nerve fibers. The development and progression of DPN is due in large part to hyperglycemia but is also affected by insulin deficiency and dyslipidemia. Although numerous biochemical mechanisms contribute to DPN, increased oxidative/nitrosative stress and mitochondrial dysfunction seem intimately associated with nerve dysfunction and diminished regenerative capacity. Despite advances in understanding the etiology of DPN, few approved therapies exist for the pharmacological management of painful or insensate DPN. Therefore, identifying novel therapeutic strategies remains paramount. Because DPN does not develop with either temporal or biochemical uniformity, its therapeutic management may benefit from a multifaceted approach that inhibits pathogenic mechanisms, manages inflammation, and increases cytoprotective responses. Finally, exercise has long been recognized as a part of the therapeutic management of diabetes, and exercise can delay and/or prevent the development of painful DPN. This review presents an overview of existing therapies that target both causal and symptomatic features of DPN and discusses the role of up-regulating cytoprotective pathways via modulating molecular chaperones. Overall, it may be unrealistic to expect that a single pharmacologic entity will suffice to ameliorate the multiple symptoms of human DPN. Thus, combinatorial therapies that target causal mechanisms and enhance endogenous reparative capacity may enhance nerve function and improve regeneration in DPN if they converge to decrease oxidative stress, improve mitochondrial bioenergetics, and increase response to trophic factors.

I. Introduction

DPN is the most prevalent complication of diabetes and often manifests as a distal, symmetric, sensorimotor neuropathy. In the United States, 26.8 million people are affected by diabetes; by the year 2030, that number is predicted to increase to approximately 35.9 million people (Shaw et al., 2010). A recent population-based study reported that more than half of patients who have type 1 or 2 diabetes develop DPN (Harati, 2007). Of these patients with DPN, 15 to 30% suffer from painful diabetic neuropathy, whereas the remainder experience a loss of sensation and numbness (Ramos et al., 2007). Clinical symptoms associated with DPN involve poor gait and balance associated with large sensory fibers and abnormal cold and/or heat sensation associated with small sensory fibers. Chronic pain associated with diabetes is represented by hyperalgesia, allodynia, paresthesias, and spontaneous pain (Gooch and Podwall, 2004; Edwards et al., 2008). Symptoms are described as tingling, “pins and needles,” burning, itching, and an abnormal sensation to pain and temperature. Over time, these symptoms may advance from the toes to the foot and up the leg, and these symptoms may occur in the fingers and hands (Zochodne, 2007).

DPN arises as a result of the degeneration of small, unmyelinated C fibers or thinly myelinated Aδ sensory fibers that mediate pain/temperature sensation. Diabetic-induced changes in C fibers lead to the development of small-fiber neuropathy, which often produces positive (painful) symptoms: algodystrophy and hyperesthesia. Progressive neurodegeneration may spontaneously resolve the neuropathic pain, but decreased response thresholds (Lennertz et al., 2011) and loss of epidermal innervation of C fibers in the feet (Beiswenger et al., 2008) can contribute to negative neuropathic symptoms such as thermal hypoalgesia. Likewise, degeneration of Aβ fibers leads to a loss of vibration and tactile sensation (Lennertz et al., 2011) with axon-myelin separation (Powell and Myers, 1983; Myers and Powell, 1984; Love et al., 1986) and eventual segmental demyelination in long-term DPN (Zochodne, 2007). Thus, deficits in both C and Aβ fiber function greatly affects the detection of noxious and non-noxious stimuli. Unfortunately, the complexity of the multiple and temporally nonuniform biochemical insults within endothelium, neurons, and Schwann cells that contribute to the sensory phenotypes associated with DPN has rendered the development of effective therapeutics difficult.

Many excellent reviews have been published that 1) describe the various biochemical insults that contribute to the pathogenesis of DPN and 2) outline treatment strategies directed at blocking either causal mechanisms or treating neuropathic pain (Leinninger et al., 2004; Vincent et al., 2004, 2009b; Pop-Busui et al., 2006; Calcutt and Backonja, 2007; Zochodne, 2007, 2008; Calcutt et al., 2008, 2009; Edwards et al., 2008; Tavakoli and Malik, 2008; Veves et al., 2008; Obrosova, 2009a; Fernyhough et al., 2010; Sivitz and Yorek, 2010; Malik et al., 2011). Thus, the intent of the current review is to briefly highlight and update many of these features and add to the discussion by proposing that both pharma-
logic and nonpharmacologic approaches that help modulate the expression and activity of cytoprotective molecular chaperones may afford a complementary tactic toward improving the management of DPN.

II. Pathogenesis of Diabetic Peripheral Neuropathy

Results from the Diabetes Control and Complication Trial (DCCT) supported the hypothesis that DPN develops as a result of increased blood glucose concentrations (hyperglycemia) (DCCT Research Group, 1988, 1993). However, more recent data seem to indicate that the development of DPN is not necessarily strictly glucocentric but also involves neuronal insulin deficiency/resistance (Kim and Feldman, 2012) and dyslipidemia (Wiggin et al., 2009). Nevertheless, the glucose-induced pathologic features of DPN are well characterized and include enhanced activity of the polyol pathway, the formation of advanced glycation end products (AGE), protein kinase C activation (PKC), increased poly(ADP-ribose) polymerase (PARP) activity, enhanced modification of proteins with N-acetyl glucosamine via the hexosamine pathway, increased inflammation, and a reduction in neurotrophic factors (Fig. 1). Many of these mechanisms contribute to increasing oxidative stress and mitochondrial dysfunction and have rightly served as focal targets in developing therapeutic interventions to improve or reverse DPN.

A. Polyol Pathway

Under normoglycemic conditions, glucose is primarily metabolized through glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation (Yagihashi et al., 2007). However, under hyperglycemic conditions, the excess intracellular glucose can be shunted through the polyol pathway, where it is reduced to sorbitol by aldose reductase (AR), which requires oxidizing NADPH to NADP⁺/H⁺. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase, which reduces NAD⁺ to NADH (Duby et al., 2004).

The polyol pathway produces excess levels of sorbitol and fructose, which can decrease expression and uptake of myo-inositol (Kato et al., 1999) and blunt activation of the Na⁺/K⁺-ATPase (Kato et al., 1999; Nayak et al., 2011). In rats with streptozocin (STZ)-induced diabetes, impaired myo-inositol metabolism through phosphoinositide synthase invoked the slowing of nerve conduction velocity, an early indicator of DPN (Greene and Mackway, 1986).

NADPH is a coenzyme that is required by glutathione reductase to convert GSSG to GSH. Because the reduction of glucose to sorbitol via AR depletes NADPH, this compromises the recycling of GSSG to GSH. Decreased GSH limits the ability of glutathione peroxidase to reduce hydrogen peroxide to water, thus increasing oxidative stress. It is noteworthy that deletion of AR prevented GSH depletion, superoxide production, and nerve conduction velocity deceleration (Ho et al., 2006). Activation of the polyol pathway is likely to be localized in peripheral nerve because AR is not very abundant in sensory neurons but preferentially localizes to Schwann cells (Jiang et al., 2006). Despite this localization, inhibiting sorbitol dehydrogenase decreased oxidative stress in cultured adult sensory neurons obtained from diabetic rats (Akude et al., 2011).

B. Advanced Glycation End-Products

AGEs are formed when amino groups of proteins react nonenzymatically with reducing sugars (Bansal et al., 2012).Dicarbonyls are AGE precursors that are formed through the oxidation of glucose to glyoxal, the breakdown of Amadori products to 3-deoxyglucosone, and methylglyoxal formation from dihydroxyacetone phosphate (Negre-Salvayre et al., 2009).

There are three general mechanisms by which the production of AGEs damages cells. First, AGEs modify the biological function of intracellular proteins and promote apoptosis (Sekido et al., 2004; Vincent et al., 2007). Second, AGEs can modify components of the extracellular matrix such as laminin and fibronectin and contribute to poor collateral sprouting and axonal regeneration associated with DPN (Duran-Jimenez et al., 2009). Third, AGEs modify plasma proteins, creating ligands that bind to the receptor for AGEs (RAGE) on macrophages, smooth muscle, vascular endothelial cells, and Schwann cells (Wada and Yagihashi, 2005). The binding of AGEs to RAGE increases the production of reactive oxygen species (ROS) (NF-κB (NF-κB), causing multiple changes in gene expression that lead to enhanced endothelial permeability, smooth muscle cell and fibroblast proliferation, extracellular matrix degradation, cytokine secretion, procoagulant effects, and apoptosis (Bierhaus et al., 2004). These changes can result in accelerated atherosclerosis, arterial stiffening, stenosis, thrombosis, and microvascular
complications (Negre-Salvayre et al., 2009). The importance of RAGE activation to DPN is underscored by observations that functional and structural abnormalities of experimental DPN, such as pain perception (Bierhaus et al., 2004), nerve conduction deficits, and axonal atrophy, are diminished in RAGE-null mice (Toth et al., 2008).

C. Protein Kinase C

PKC comprises a family of enzymes that phosphorylate numerous target proteins. The activity of many PKC family members depends on Ca\(^{2+}\) ions, phosphorylserine, and diacylglycerol; chronic hyperglycemia elevates diacylglycerol levels. In general, the contributions of aberrant PKC activation to DPN seem to center on affecting nerve blood flow and improving nerve conduction velocity deficits (Obrosova et al., 2009a). In vitro, PKC activation can lead to NF-κB activation, overexpression of plasminogen activator inhibitor-1, the expression of vascular endothelial growth factor (Madonna and De Caterina, 2011), along with reductions in nitric oxide production in Zucker fatty rats (Bohlen, 2004). These pathological changes can alter vasoconstriction and capillary permeability (Edwards et al., 2008). PKC activation can lead to NF-κB activation, overexpression of plasminogen activator inhibitor-1, the expression of vascular endothelial growth factor (Madonna and De Caterina, 2011), along with reductions in nitric oxide production in Zucker fatty rats (Bohlen, 2004). These pathological changes can alter vasoconstriction and capillary permeability (Edwards et al., 2008). PKCβ is also involved in the mechanism, leading to reduced Na\(^{+}/K\(^{+}\)\)-ATPase activity, resulting in decreased nerve conduction velocities and nerve regeneration (Lehning et al., 1994). Treatment with an inhibitor of PKCβ improved MNCV, normalized nerve blood flow, and restored Na\(^{+}/K\(^{+}\)\)-ATPase activity in STZ-induced diabetic rats (Cameron and Cotter, 2002).

D. Poly(ADP-ribose) Polymerase Pathway

PARP is a ubiquitous nuclear DNA repair enzyme that cleaves NAD\(^{+}\) to produce ADP-ribose residues that can attach to proteins. PARP has been shown to cause and to be activated by oxidative stress (Obrosova et al., 2005). Enhanced PARP activity depletes NAD\(^{+}\), leading to energy failure, increased oxidative stress, and an inhibition of glyceraldehyde 3-phosphate dehydrogenase (Obrosova et al., 2005; Pacher and Szabo, 2008; Negi et al., 2010a,b). PARP has been implicated in nerve conduction velocity deficits, neurovascular dysfunction, thermal and mechanical hyper- and hypalgesia, mechanical allodynia, and myelinated fiber loss (Obrosova et al., 2005, 2009a; Pacher and Szabo, 2008; Homs et al., 2011; Stavniichuk et al., 2011; Dieckmann et al., 2012). Moreover, an increase in poly(ADP-ribosylated) proteins has been reported after 4 weeks of diabetes in rat sciatic nerve and in cultured human Schwann cells (Obrosova et al., 2005). Although the identification of specific ADP-ribosylated proteins and their contribution to the progression of DPN remains unknown, the fact that PARP-1 knockout mice do not develop small-fiber neuropathy supports an important role of PARP activation in the development of DPN (Obrosova et al., 2004, 2008).

E. Hexosamine Pathway

During normal glucose metabolism, approximately 3% of total glucose is diverted into the hexosamine pathway via fructose-6-phosphate (F-6-P) (Marshall et al., 1991), and the magnitude of this shunting can increase in hyperglycemia. Increased PARP activity depletes NAD\(^{+}\), and to be activated by oxidative stress (Obrosova et al., 2005). Enhanced PARP activity depletes NAD\(^{+}\), and to be activated by oxidative stress (Obrosova et al., 2005). Enhanced PARP activity depletes NAD\(^{+}\), leading to energy failure, increased oxidative stress, and an inhibition of glyceraldehyde 3-phosphate dehydrogenase (Obrosova et al., 2005; Pacher and Szabo, 2008; Negi et al., 2010a,b). PARP has been implicated in nerve conduction velocity deficits, neurovascular dysfunction, thermal and mechanical hyper- and hypalgesia, mechanical allodynia, and myelinated fiber loss (Obrosova et al., 2005, 2009a; Pacher and Szabo, 2008; Homs et al., 2011; Stavniichuk et al., 2011; Dieckmann et al., 2012). Moreover, an increase in poly(ADP-ribosylated) proteins has been reported after 4 weeks of diabetes in rat sciatic nerve and in cultured human Schwann cells (Obrosova et al., 2005). Although the identification of specific ADP-ribosylated proteins and their contribution to the progression of DPN remains unknown, the fact that PARP-1 knockout mice do not develop small-fiber neuropathy supports an important role of PARP activation in the development of DPN (Obrosova et al., 2004, 2008).

F. Oxidative/Nitrosative Stress and Mitochondrial Dysfunction

Chronic or acute hyperglycemia can induce oxidative stress by overloading the flow of reducing equivalents from glycolysis and the TCA cycle into oxidative phosphorylation. In hyperglycemic stressed endothelial cells, the shunting of excess glucose into glycolysis and the TCA pathway increases NADH and FADH\(_2\). This contributes to producing a hyperpolarized membrane potential as a result of the pumping of excess proteins across the inner mitochondrial membrane (Nishikawa et al., 2000). This slows electron transport and can increase the production of superoxide anion produced at complex I (Treberg et al., 2011) and complex III of the electron transport chain. Superoxide produced at complex I is directed primarily to the mitochondrial matrix, whereas that generated at complex III is more equally distributed between the mitochondrial matrix and intermembrane space (Sivitz and Yorek, 2010). Thus, both sites of superoxide production may contribute to oxidative damage of mitochondrial proteins.
It has been suggested that increased mitochondrial superoxide production may underlie the hyperglycemia-induced increases in polyol synthesis, PKC activation, protein GlcNAcylation, AGE formation, and the development of diabetic complications (Nishikawa et al., 2000). The elegant simplicity of the hypothesis is attractive and probably applicable to endothelium. For example, a complex I-dependent increase in mitochondrial superoxide was observed in epineurial arterioles of sciatic nerve from STZ-diabetic rats (Coppey et al., 2003). However, it is unlikely to account for all aspects of enhanced oxidative stress in diabetic nerve. In adult sensory neurons, prolonged diabetes (22 weeks) was associated with depolarization of the inner mitochondrial membrane and was not accompanied by an increase in superoxide production (Huang et al., 2003; Akude et al., 2011). Nonetheless, diabetic sensory neurons still exhibit enhanced levels of 4-hydroxy-2-nonenal, a product of lipid peroxidation (Akude et al., 2011). Regardless of whether enhanced superoxide generation is a central unifying mechanism in the pathophysiology of diabetic complications, its formation is critical to increasing protein nitrination, via the formation of peroxynitrite (Ishiiropoulos and Beckman, 2003). Although it is unclear whether peroxynitrite is actually produced within mitochondria, protein nitrination is increased in sensory neuron cell bodies and peripheral nerve of diabetic animals (Obrosova et al., 2005; Vareniuk et al., 2007). It is noteworthy that the inducible form of nitric-oxide synthase seems more critical to the development of neuropathic symptoms than the neuronal isoform of this enzyme (Vareniuk et al., 2008, 2009). Although nitrosative stress contributes to DPN, it should be a priority to identify the role of specific nitrosylated proteins in the development and/or progression of DPN.

A recent review has extensively summarized the effect of diabetes-induced oxidative stress on mitochondrial function (Sivitz and Yorek, 2010). Numerous reports using adult sensory neurons or dorsal root ganglia isolated from diabetic rats support that prolonged diabetes can decrease inner mitochondrial membrane potential and respiratory chain activity (Srinivasan et al., 2000; Huang et al., 2003; Huang et al., 2005; Chowdhury et al., 2010, 2011). It is noteworthy that in-depth analysis of mitochondrial bioenergetics using intact adult sensory neurons isolated from diabetic rats (4 months of diabetes) (Chowdhury et al., 2012) or mice (6 months of diabetes) (Urban et al., 2012b) supports the theory that diabetes also decreases spare respiratory capacity. Because spare respiratory capacity is indicative of the bioenergetic limit at which a cell is functioning (Sansbury et al., 2011), a decrease in spare respiratory capacity limits the dynamic range available to respond to environmental challenges. This renders neurons more susceptible to stress because they cannot increase energy demand sufficiently to match environment needs (Nicholls et al., 2007; Choi et al., 2009). Not surprisingly, diabetes-induced increases in mitochondrial workload (Chowdhury et al., 2012; Zhang et al., 2012) also correlate with alterations in the mitochondrial proteome of sensory neurons. We recently demonstrated that a decrease in mitochondrial respiration of dorsal root ganglia isolated from diabetic rats (22 weeks of diabetes) correlated with a decrease in proteins linked to oxidative phosphorylation, ubiquinone biosynthesis, the TCA cycle, and antioxidant protection (i.e., MnSOD) (Akude et al., 2011; Chowdhury et al., 2011). It is noteworthy that insulin therapy reversed many of these changes in the mitochondrial proteome, indicating its highly dynamic nature (Akude et al., 2011). Although it is unclear how diabetes may directly alter the mitochondrial proteome (i.e., enhanced degradation versus decreased synthesis), quantitative proteomic analysis of protein translation indicated that hyperglycemic stress can negatively affect the translation of numerous mitochondrial proteins in primary cultures of embryonic sensory neurons (Zhang et al., 2012).

G. Inflammation, Lipid Mediators, and Dyslipidemia

Proinflammatory cytokines contribute to the pathogenesis and/or maintenance of neuropathic pain. Injury to peripheral nerves results in the production of cytokines that originate from resident and recruited lymphocytes, macrophages, neurons, and Schwann cells (Yasuda et al., 2003). Patients with both type 1 and 2 diabetes exhibit elevated blood levels of tumor necrosis factor-α (TNF-α), an inflammation promoting cytokine (Gonzalez-Clemente et al., 2005; Purwata, 2011). In these patients with diabetes, the plasma levels of TNF-α correlated with the severity of perceived pain (Purwata, 2011). The relevance of increased TNF-α levels to DPN is supported by both pharmacologic and genetic evidence. Infliximab is an FDA-approved monoclonal antibody that binds soluble TNF, and its administration decreased plasma TNF-α levels and attenuated nerve conduction deficits in diabetic mice (Yamakawa et al., 2011). Moreover, diabetic TNF-α knockout mice were resistant to developing DPN (Yamakawa et al., 2011). It is noteworthy that pharmacologically inhibiting cyclooxygenase 2 (COX-2) in diabetic mice prevented an increase in nerve levels of TNF-α and activation of NF-κB (Kellogg et al., 2007). Likewise, diabetic COX-2 knockout mice were resistant to developing DPN and showed no increase in nerve TNF-α levels (Kellogg and Popp-Busui, 2005; Kellogg et al., 2007). Inhibiting COX-2 also prevented spinally mediated hyperalgesia in diabetic rats and through inhibiting AR, blocked COX-2 activation, whether TNF-α levels were decreased was not examined (Ramos et al., 2007). These data would support that the production of TNF-α is downstream of arachidonic acid metabolism.

Finally, it should be noted that the role of TNF-α in the onset of DPN may vary with disease severity, duration, and/or species. For example, TNF-α levels and
NF-κB signaling were decreased in dorsal root ganglia (DRG) obtained from rats that had a relatively moderate level of diabetes for 5 months (Saleh et al., 2011). Likewise, despite an increase in the expression of COX-2, TNF-α levels were decreased in the sciatic nerve of rats that manifested a tactile allodynia after 4 weeks of diabetes (Jolivalt et al., 2009). These data suggest that at least in the diabetic rat model, early sensory neuropathy is not associated with the enhanced production of TNF-α that is apparent in longer term and more severely diabetic animals. Indeed, numerous genes associated with the gene ontology category of inflammatory response were up-regulated in sural nerve biopsies from patients classified as having progressive DPN (Hur et al., 2011). How these mediators may contribute to ultrastructural changes in humans with minimal but progressive neuropathy remains unclear (Malik et al., 2005).

12/15-Lipoxygenases convert arachidonic acid to 12/15-hydroxyeicosatetraenoic (HETE) acids. These inflammatory lipid mediators are increased in diabetic nerve and spinal cord and enhance oxidative/nitrosative stress (Stavniichuk et al., 2010). It is noteworthy that inhibiting AR decreased formation of 12-HETE in sciatic nerve but not spinal cord (Stavniichuk et al., 2012), suggesting that the lipid metabolite may be produced preferentially in Schwann cells. Furthermore, neither p42/44 nor p38 MAPKs were activated in sciatic nerve of diabetic 12/15-lipoxygenase knockout mice. Given the localization of AR to Schwann cells and the role of aberrant p42/p44 MAPK in promoting demyelination (Harrissingh et al., 2004; Syed et al., 2010; Napoli et al., 2012), these data suggest that the coordinated actions of the polyol pathway and 12-HETE production may increase the degradation of myelin in Schwann cells via enhanced activation of p42/p44 MAPK. Consistent with this possibility, larger myelinated fibers in tibial nerve are spared from myelin thinning in diabetic 12/15-lipoxygenase-deficient mice, but the gene deletion did not prevent the loss of small unmyelinated fibers innervating the epidermis (Obrosova et al., 2010).

A growing body of evidence supports expanding the focus on glucocentric mechanisms in the development and progression of DPN to also encompass dyslipidemia (Vincent et al., 2009b). Evidence supporting this premise has been gathered from human cohorts (Wiggin et al., 2009) and by feeding mice a high-fat diet, which promoted DPN even in the absence of elevating blood glucose level (Obrosova et al., 2007b; Vincent et al., 2009a). Remarkably, a recent report indicates that superimposing dyslipidemia on a diabetic background can differentially affect sensory modalities associated with DPN. Diabetic C57BL/6 mice given a standard chow exhibited a mechanical hypoalgesia (an insensate neuropathy), whereas diabetic mice placed on a high-fat diet exhibited a mechanical allodynia (a painful neuropathy) (Guilford et al., 2011). On the other hand, the high-fat diet did not significantly worsen the thermal hypoalgesia that was manifested in the diabetic mice. The effect of the high-fat diet on promoting a mechanical allodynia may be related to an increase in 12/15-lipoxygenase activity because an ethanolic extract from the plant *Artemisia dracunculus* L. helped normalize these measures (Watcho et al., 2010). These results suggest that a high-fat diet may differentially affect metabolic functions in myelinated versus unmyelinated fibers. Given the complex interactions between glucose and lipid metabolism, as well as nerve function and psychosensory behaviors, systems biology approaches will undoubtedly prove useful in dissecting how the up-/down-regulation of gene networks by diabetes and dyslipidemia may differentially contribute to the progression and severity of DPN (Wiggin et al., 2008; Hur et al., 2011; Pande et al., 2011).

**H. Growth Factors**

Neurotrophic factors are molecules that develop and maintain the nervous system by promoting the growth and/or survival of neurons. The neurotrophin family of growth factors includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5. Glial cell-derived neurotrophic factors (GDNF) form a second family and include GDNF, neurturin, artemin, and persephin. Each neurotrophic factor regulates neuronal function and supports the growth and survival of distinct groups of neurons (Boucher and McMahon, 2001). Likewise, members of the neuregulin family of growth factors are important for the survival of Schwann cells (Syroid et al., 1996) and regulate both the formation (Taveggia et al., 2005; Nave and Salzer, 2006; Syed et al., 2010) and degeneration (Zanazzi et al., 2001; Syed et al., 2010) of the myelin sheath.

A deficiency in NGF and NT-3 has long been characterized in both tissue and serum in DPN (Faradji and Sotelo, 1990; Hellweg and Hartung, 1990; Fernyhough et al., 1998). Diabetes also reduces anterograde and retrograde axonal transport of BDNF, NGF, and NT-3 in peripheral nerves (Hellweg et al., 1994; Fernyhough et al., 1998; Mizisin et al., 1999). Loss of neurotrophic support can affect fiber morphology because intrathecal delivery of NGF or NT-3 improved myelinated fiber innervation in the dermal footpad of diabetic mice (Christianson and Salter, 2006). Likewise, unmyelinated, nonpeptidergic fibers were increased after intrathecal administration of GDNF (Akkina et al., 2001), whereas intramuscular GDNF gene therapy improved myelination and neuromuscular levels in the sciatic nerve of diabetic rats (Liu et al., 2009). In addition, GDNF may be useful in ameliorating autonomic neuropathy associated with poor gastrointestinal motility (Anitha et al., 2006).

Although chronic hyperglycemia is considered the major trigger in the pathogenesis of DPN (DCCT Research Group, 1993), patients with impaired glucose tolerance (prediabetes) also develop DPN (Smith and Singleton, 2008). In an STZ rat model, altered mechanical sensitivity occurred before the development of hyperglycemia,
when blood insulin levels fell below 2 ng/ml; the onset of hyperglycemic-induced DPN occurred when insulin levels decreased to less than 0.3 ng/ml (Romanovsky et al., 2006). Thus, impaired insulin signaling or insulin deficiency in peripheral neurons may also contribute to DPN. Insulin is important for general neuronal function (Kim and Feldman, 2012; Urban et al., 2012a), and insulin receptors are abundantly expressed in neuronal cell bodies in the DRG and peripheral axons innervating the epidermis (Sugimoto et al., 2000, 2002; Toth et al., 2006; Guo et al., 2011). It is noteworthy that neuronal insulin receptors are increased after physical injury of peripheral nerves (Toth et al., 2006) and in diabetes (Guo et al., 2011). At doses insufficient to alter hyperglycemia, local injections of insulin into the hindpaw footpad of diabetic mice improved nerve fiber density and mechanical sensation (Guo et al., 2011). Likewise, intranasal insulin administration reduced several physiological indices of DPN and increased sensory nerve fibers in the plantar footpads in the absence of correcting the systemic hyperglycemia (Francis et al., 2009).

Insulin deficiency may alter neuronal function in diabetic sensory neurons by decreasing mitochondrial respiration because insulin enhanced respiratory activity and inner mitochondrial membrane potential in a phospha-tidylinositol-3-kinase-dependent manner (Huang et al., 2003, 2005; Chowdhury et al., 2010). It is also notable that insulin-dependent mitochondrial production of H$_2$O$_2$ can enhance insulin receptor phosphorylation in cerebellar granule neurons (Storozhevykh et al., 2007). However, diabetic sensory neurons are less responsive to producing ROS, even when using the potent superoxide inducer antimycin A (Zherebitskaya et al., 2009; Chowdhury et al., 2010). Although it remains unclear whether the increase in insulin receptor phosphorylation by H$_2$O$_2$ is critical for the efficacy of insulin, these results suggest that a point may exist at which insulin therapy may no longer improve mitochondrial function if insulin-induced H$_2$O$_2$ production is compromised (Cheng et al., 2010). Along this line, the electrophysiological deficits of DPN were less responsive to insulin as the disease progressed (Szilvássy et al., 2012).

The response of Schwann cells to growth factors may also be affected in diabetic nerve. Neuregulin-1 forms a family of several gliotrophic factors (Esper et al., 2006) that bind to Erb B2 receptors, which preferentially localize to Schwann cells (Grinspan et al., 1996). Neuregulins play a complex role in the regulation of myelination because they may both promote myelination and induce demyelination in a concentration-dependent manner (Syed et al., 2010). After axotomy, increases in neuregulin levels may contribute to Wallerian degeneration by activating Erb B2 receptors (Guertin et al., 2005). In cultured neonatal rat Schwann cells, hyperglycemia stimulated neuregulin-induced Erb B2 activation and increased thymidine uptake (Tan et al., 2003). On the other hand, a separate report found no effect of hyperglycemia on Erb B2 signaling and a decrease in neuregulin-induced mitogenesis (Gumy et al., 2008). Although the underlying reason for these discrepant findings is unclear, changes in Erb B2 activity may indeed contribute to altered nerve function in DPN. In diabetic mice, Erb B2 phosphorylation increased in sciatic nerve, and this was correlated with a decrease in MNCV and the induction of a sensory hypoalgesia (McGuire et al., 2009). It is noteworthy that treating diabetic C57BL/6 mice with an Erb B2 inhibitor, 4-phenethylamino-6-(hydroxyl)phenyl-7H-pyrrolo(2,3-d)pyrimidine (PKI-166) or erlotinib, attenuated the electrophysiological deficits and improved mechanical but not thermal hypoalgesia. Because thermal sensitivity is mediated primarily via unmyelinated C fibers (Julius and Basbaum, 2001), these data indicate that myelinated fibers may be more sensitive to pathological activation of Erb B2. In this regard, hyperglycemia increased the extent of neuregulin-induced demyelination in myelinated Schwann cell-sensory neuron cocultures (Yu et al., 2008). However, the effect of diabetes on the expression of various neuregulin-1 isoforms and whether they contribute to myelin thinning in peripheral nerve remains to be determined.

### III. Management of Diabetic Peripheral Neuropathy

#### A. Targeting Hyperglycemia

Strict glycemic control is the only proven method available that prevents the development of DPN and/or slows the progression of this disease (Tesfaye et al., 2005, 2011). This is mainly achieved with the use of oral antidiabetics and insulin in conjunction with lifestyle changes in diet and exercise. Although modulating incretin expression is a new approach toward managing blood glucose levels, increasing incretin levels may directly improve DPN in type 1 diabetes, even without affecting blood glucose levels. Receptors for the incretin, glucagon-like peptide 1, localize to axons and Schwann cells of sciatic nerve (Jolivalt et al., 2011). Treating diabetic rats with exenatide, a glucagon-like peptide-1 mimetic, improved MNCV and intraepidermal nerve fiber density without improving plasma insulin and blood glucose (Jolivalt et al., 2011). Likewise, dipeptidyl peptidase IV inhibitors are a new class of antidiabetic agents that increase the half-life of endogenous incretins. Because a vildagliptin analog also improved DPN in diabetic rats without affecting blood glucose levels (Bianchi et al., 2012), incretins are likely to have extrapancreatic effects that are directly neurotrophic (Perry et al., 2002).

#### B. Targeting Casual Mechanisms

1. **Aldose Reductase Inhibitors.** Due to the numerous negative effects of increased AR activity during chronic hyperglycemia, the inhibition of this enzyme remains an attractive strategy for treating DPN (Oates, 2008). Aldose reductase inhibitors inhibit tissue accumulation of
sorbitol and fructose by reducing the flux of glucose through the polyol pathway and are represented by three chemical classes; acetic acid compounds, spirohydantoins, and a succinimide (Schemmel et al., 2010), although new agents also continue to be evaluated (Macari et al., 2011; Rapposelli et al., 2011).

Alrestatin, epalrestat, ponalrestat, tolrestat, zenarestat, and zopolrestat represent the acetic acid compounds. Sorbinil and fiderostat are spirohydantoins, and ranirestat is a succinimide. Although all these agents have shown efficacy in animal models of DPN, epalrestat is the only AR inhibitor that has been approved to treat DPN and has been available in Japan since 1992 (Schemmel et al., 2010). In patients with diabetes treated with ranirestat for 12 weeks, the drug dose dependently inhibited sorbitol and fructose levels in the sural nerve (Bril and Buchanan, 2004), and a 48-week extension of this study revealed an improvement in SNCV by ≥1 m/s relative to baseline and compared with placebo (Bril and Buchanan, 2006). Unfortunately, in phase 3 testing, 52 weeks of ranirestat treatment (20 and 40 mg/day) failed to improve summed SNCV, possibly related to an unexpected improvement in SNCV in the placebo group (Bril et al., 2009). However significant improvements in MNCV were observed and the drug was well tolerated, suggesting that a more refined trial design may demonstrate efficacy in the primary endpoint of summed SNCV (Bril et al., 2009).

2. Blocking Advanced Glycation End-Product Formation and Activation of the Receptor for Advanced Glycation End-Products. Several experimental and/or clinical studies have examined agents that reduce/inhibit AGE formation, cleave AGEs, or block RAGE. Aminoguanidine was the first AGE inhibitor studied and prevents AGE formation by reacting with carbonyl groups of reduced sugars (Sugimoto et al., 2008). Double-blind, multidose, placebo-controlled, randomized clinical trials in patients with type 1 and 2 diabetes with diabetic nephropathy showed no reduction in the nephropathy progression (Freedman et al., 1999; Bolton et al., 2004). Although aminoguanidine did slow the development of retinopathy in these patients, clinical trials have been discontinued because of adverse effects, such as gastrointestinal disturbances and liver function test abnormalities (Bolton et al., 2004).

An alternative strategy for decreasing AGEs is to decrease methylglyoxal, a major dicarbonyl glucose metabolite that modifies arginine residues of proteins. In this regard, diabetic rats treated with thiamine or benfotiamine (also see section B.4) showed decreased levels of protein glycation (Karachalias et al., 2010). Likewise, Xue et al. (2011) suggest that up-regulating glyoxalase 1, which metabolizes methylglyoxal, can decrease methylglyoxal-derived protein adducts. Glyoxalase activity may also be a potential biomarker for susceptibility to DPN. BALB/cByJ mouse express a 10-fold greater level of glyoxalase 1 than BALB/cJ mice and were resistant to developing an insensate neuropathy and loss of intraepidermal nerve fibers compared with the diabetic BALB/cJ mice (Jack et al., 2012).

Another approach to modulate the effects of AGEs is to prevent their binding to RAGE. This is achieved with a soluble form of the extracellular ligand-binding domain of RAGE (sRAGE) and/or anti-RAGE antibodies. Similar to diabetic RAGE knockout mice, wild-type mice treated with sRAGE show a diminished extent of diabetes-related complications (Sakaguchi et al., 2003; Wendt et al., 2003). In diabetic rodents, sRAGE treatment normalized thermo-nociception and corrected neuronal deficits (Bierhaus et al., 2004). The presence of endogenous sRAGE has also been identified in the blood. In patients with diabetes who have DPN, plasma levels of sRAGE were decreased by 59 and 77% compared with patients with diabetes who did not have DPN and healthy patients without diabetes, respectively (El-Mesallamy et al., 2011). Although sRAGE is in clinical trials for other disorders, no current trials were listed for its use in DPN in early 2012 (http://www.clinicaltrials.gov).

3. Protein Kinase C Inhibitors. Ruboxistaurin (RBX, also known as LY333531 or Arxaxant) is a bisindolylmaleimide that selectively inhibits PKC-β1 and PKC-β2 (Geraldes and King, 2010). RBX has successfully reduced the development of diabetic retinopathy and nephropathy (Beckman et al., 2002; Tuttle et al., 2005; Aiello et al., 2006), but it has not been as successful in the setting of DPN (Vinik et al., 2005). However, in a subset of patients with less severe DPN, RBX did significantly improve neuropathic symptoms and nerve function compared with placebo. Nonetheless, the status for advancing the approval of RBX for use in DPN is unclear.

4. Hexosamine Pathway. Currently, pharmacologic modulation of the hexosamine pathway is primarily indirect. Thiamine (vitamin B1) is a water-soluble vitamin that serves, after phosphorylation, as a coenzyme for transketolase. Transketolase plays a fundamental role in intracellular glucose metabolism by shifting excess F-6-P from glycolysis into the pentose phosphate pathway, thereby preventing activation of the hexosamine pathway and decreasing methylglyoxal formation as described above (Beltramo et al., 2008). Patients with diabetes show a high prevalence of thiamine and transketolase deficiency (Thornalley et al., 2007; Alam et al., 2011), and high doses of thiamine in combination with pyridoxine (vitamin B6) improved neuropathic symptoms in patients with diabetes who have DPN (Abbas and Swai, 1997).

Benfotiamine is a lipid-soluble thiamine derivative with improved bioavailability (Beltramo et al., 2008; Balakumar et al., 2010). In the Benfotiamine in Diabetic Polyneuropathy pilot study (Haupt et al., 2005), benfotiamine (400 mg/day for 3 weeks) improved painful neuropathic symptoms; similar results were seen in a follow-up phase 3 clinical trial, (600 mg/day benfotiamine).
for 6 weeks) (Stracke et al., 2008). Benfotiamine was well tolerated with no side effects in both clinical trials and is available as a dietary supplement in the United States. As mentioned above, any benefits of benfotiamine are likely to include modulation of AGEs as well.

5. Antioxidants, α-Lipoic Acid (Thioctic Acid). Increasing antioxidant defense is a logical therapeutic approach for treating DPN and α-lipoic acid (LA) is among the better studied antioxidants in humans. Oral administration of LA (minimum of 600 mg/day) reduced neuropathic symptoms associated with DPN by 50% from baseline in the SYDNEY 2 trial (Ziegler et al., 2006). However, compared with results from subjects in the control group, this reduction in symptoms falls below the clinically relevant threshold of 30% (Mijnhout et al., 2010). The recent Neurological Assessment of Thioctic Acid in Neuropathy (NATHAN) 1 trial was a 4-year treatment (600 mg/day) of patients with diabetes and mild-to-moderate DPN (Ziegler et al., 2011). LA did not significantly improve nerve conduction attributes but did improve some scores of small-fiber neuropathy and muscular function. It is important to note that the lack of efficacy in the NATHAN 1 trial seems to arise from the lack of further decline of nerve conduction deficits in the placebo-treated group during the study (Ziegler et al., 2011), an inherent challenge to trial design in DPN (Dyck et al., 2007). LA is approved for treating DPN in Germany (Gooch and Podwall, 2004) and is commercially available as a dietary supplement in the United States.

6. Growth Factors. The use of growth factors in treating DPN has been extensively explored (Leinninger et al., 2004; Calcutt et al., 2008). NGF, BDNF, and NT-3 have been assessed in various levels of clinical trials of DPN with limited success. NGF therapy has advanced the furthest but showed no efficacy in a large, multicenter phase III trial (Apfel, 2002). In addition, a recent proof-of-concept trial examining the analgesic effects of tanezumab, a monoclonal antibody against NGF, was halted in 2010 for potential safety issues (http://www.clinicaltrials.gov/ct2/show/NCT01087203).

C. Targeting Painful Symptoms

Controlling pain is one of the most difficult management issues encountered when treating patients who have DPN. A number of drugs have shown efficacy at managing neuropathic pain in randomized clinical trials. Unfortunately, there are few FDA-approved treatments and no standardized algorithms for treating neuropathic pain associated with DPN.

1. Tricyclic Antidepressants. Tricyclic antidepressants (TCAs) inhibit the reuptake of noradrenaline and/or serotonin, which modulates pain transmission within the central nervous system. Amitriptyline, imipramine, and desipramine are considered the first-line treatment for DPN, although large, controlled trials seem to be lacking (Veves et al., 2008). Nonetheless, a meta-analysis revealed that one in three patients with diabetes who have DPN that is treated with TCAs achieved a 50% reduction in neuropathic pain (Mendell and Sahenk, 2003). On the other hand, duloxetine (Cymbalta) is a serotonin-norepinephrine reuptake inhibitor that relieves pain by increasing synaptic availability of serotonin and norepinephrine in the descending inhibitory pathway (Smith and Nicholson, 2007). Duloxetine is the only antidepressant approved by the FDA for the treatment of DPN (Raskin et al., 2005).

2. Anticonvulsants. Anticonvulsants were originally developed to prevent seizures, and because neuronal hyperexcitability is associated with neuropathic pain, it is believed they may be used for treating this debilitating symptom of DPN. Anticonvulsants regulate neuronal hyperexcitability by blocking calcium and/or sodium channels, through the enhancement of inhibitory GABAergic neurotransmission and inhibition of glutamatergic neurotransmission (Sullivan and Robinson, 2006).

Gabapentin (Neurontin) and pregabalin (Lyrica) are the two anticonvulsants used most frequently to treat neuropathic pain. These agents are structurally related compounds and are derivatives of the inhibitory neurotransmitter GABA; they do not have a direct pharmacological effect on GABA uptake or metabolism (Spruce et al., 2003). The α2-δ site of the voltage-gated calcium channel is the target of these agents. They reduce synaptic neurotransmitter release into hyperexcited neurons by diminishing calcium intake at nerve terminals (Finnerup et al., 2010).

Gabapentin at a dose of 1800 mg/day is effective in treating DPN (Backonja et al., 1998). Pregabalin is considered a successor to gabapentin and is more rapidly absorbed (1 versus 3–4 h) and has a higher bioavailability (90 versus 33–66%) than gabapentin (Piyapolrungroj et al., 2001). In the clinical setting, pregabalin (300–600 mg/day) effectively alleviates pain associated with DPN (Rosenstock et al., 2004; Tolle et al., 2008), and it is the only anticonvulsant approved for the treatment of DPN in the United States and Europe (Tzellos et al., 2010).

3. Opioids and Topical Analgesics. Opioids are effective analgesics used for treating both acute and chronic pain. Several opiates, such as controlled and sustained-released oxycodone, tapentadol, and tramadol, have shown clinical efficacy in relieving painful DPN (Harati et al., 1998; Gimbel et al., 2003; Watson et al., 2003; Schwartz et al., 2011; Yao et al., 2012). The most common side effects that occur with opioid use are constipation, dizziness or vertigo, dry itchy skin, nausea, somnolence or drowsiness, and vomiting (Furlan et al., 2006). Although opioids may be effective in treating DPN, the development of tolerance with a potential for dependence may occur with repeated and prolonged exposure. Consequently, other therapeutic agents are prescribed first; however, when these therapies fail to provide sufficient pain relief, opioids may be the only choice.
Topical treatments offer several therapeutic advantages, including lack of drug interactions, minimal side effects, and no need for dose titration (Tesfaye et al., 2011). However, few randomized control trials have been designed that explore the use of topical treatments as a therapy for treating neuropathic pain in DPN. Topical lidocaine is a voltage-gated sodium channel blocker that has been recommended as a first line treatment for localized neuropathic pain. A systematic review reported that 5% lidocaine medicated patches are effective in reducing painful DPN, and these patients experience a greater improvement in quality of life (Wolff et al., 2010). Side effects include burning sensation, elevated aspartate aminotransferase levels and blood pressure, headache, muscle spasms, and tingling sensation. However, compared with other agents used to reduce symptoms associated with painful DPN, 5% lidocaine medicated patches are associated with fewer and less clinically significant adverse events (Wolff et al., 2010). The FDA has approved these patches.

Capsaicin is a natural product isolated from red chili peppers. Its topical application depletes substance P from sensory nerves and has shown promising effects on painful DPN (Zhang and Li Wan Po, 1994). The Capsaicin Study Group (1992) evaluated the efficacy of capsaicin for treating DPN in an 8-week, double-blind, placebo-controlled study. They concluded that topical capsaicin is effective for treating painful symptoms associated with DPN. In addition, patients exhibited improvements in daily activities and an overall enhancement in their quality of life (Capsaicin Study Group, 1992). Although a patch containing 8% capsaicin has yielded encouraging results in treating peripheral neuropathic pain (Peppin et al., 2011), a medical concern is that its application can result in denervation of the epidermis, which can be exacerbated in patients with diabetes and neuropathy (Polydefkis et al., 2004).

D. Chaperoning Diabetic Stress—Targeting Tolerance?

It is clear from the discussion above that the efficacy of many agents in preventing or reversing experimental neuropathy in rodents has not readily translated to aiding the management of human DPN. It is likely that this represents limitations of the animal models in serving as reliable surrogates for both functional and morphologic deficits seen in human DPN, but this is not necessarily the sole problem (Calcutt et al., 2009). Another confounding factor in identifying effective treatments is that the contribution of these targets/pathways to the progression of DPN differs between patients and does not occur with temporal and/or biochemical uniformity. Thus, with necessary attention to good glycemic control, combinatorial therapies targeting multiple glucose-mediated insults may prove the most beneficial. However, a parallel and/or complementary approach to help counteract glucotoxicity is up-regulating endogenous cyto-protective responses via pharmacologic modulation of molecular chaperones.

1. Controlling the Biological Outcome of Inhibiting the 90-kDa Heat-Shock Protein: Dissociating Cytotoxicity from Cytoprotection Is Paramount to Attain Specific Therapeutic Efficacies. Molecular chaperones, such as the 70- and 90-kDa heat-shock proteins (Hsp70 and Hsp90), are essential for the folding of nascent polypeptides into their biologically active structures and for refolding aggregated and denatured proteins that may occur upon cell stress (Mayer and Bukau, 2005; Peterson and Blagg, 2009). Energy derived from hydrolysis of ATP by the intrinsic N-terminal ATPase activity of Hsp90 is important in the conformational maturation of “client proteins” that form a stabilized complex with homodimerized Hsp90. Inhibiting Hsp90 interferes with protein maturation, which leads to degradation of Hsp90 client proteins (Powers and Workman, 2007). Because many oncoproteins are Hsp90 clients, N-terminal Hsp90 inhibitors are attractive as potential chemotherapeutic agents because they can promote cytotoxicity via oncoprotein degradation (Bishop et al., 2007; Taldone et al., 2009). An important aspect of N-terminal Hsp90 inhibitors in cancer therapy is that the drugs selectively inhibit Hsp90 and induce client protein degradation in malignant versus normal cells (Chiosis et al., 2003; Kamal et al., 2003; Luo et al., 2008). Although this selectivity aids the clinical efficacy of N-terminal Hsp90 inhibitors, their use has been hampered because induction of client protein degradation and cytotoxicity can occur at drug concentrations that also activate an antagonistic aspect of Hsp90 biology, induction of the cytoprotective heat-shock response (HSR) (Fig. 2).

Hsp90 binds the transcription factor heat-shock factor 1 (HSF1) and inhibits its transactivating capacity (Ben naroch, 2011). Upon exposure to stress or Hsp90 inhibitors, HSF1 dissociates, trimerizes, undergoes phosphorylation, and translocates to the nucleus to up-regulate antioxidant genes and a variety of chaperones: the heat-shock response. Unfortunately, because chaperones can function as prosurvival factors that facilitate refolding of the oncoproteins, HSR induction antagonizes the desired cytotoxicity when treating malignancies. On the other hand, although induction of the HSR interferes with their chemotherapeutic potential, stimulating this aspect of Hsp90 biology has potential utility for treating neurodegenerative diseases associated with protein misfolding: HSR induction decreases protein aggregation.

N-terminal Hsp90 inhibitors have been shown to decrease τ protein aggregation in Alzheimer disease models (Dickey et al., 2007; Luo et al., 2007) and improve motor function in spinal and bulbar muscular atrophy (Waza et al., 2005). Although a similar selectivity exists for the use of N-terminal inhibitors in treating neurodegenerative diseases (Dickey et al., 2007), this selectivity does not circumvent the issue related to dissociating client protein degradation from induction of the HSR. Now, the inverse caveat...
exists; despite being neuroprotective, induction of client protein degradation may produce cytotoxicity. Thus, developing a highly effective Hsp90 inhibitor for treating neuronal decline requires establishing a sufficient therapeutic window that avoids client protein degradation, which antagonizes a cytoprotective HSR. Along this line, Hsp90 also contains a C-terminal ATP binding domain that binds the bacterial DNA gyrase inhibitor, novobiocin (Marcu et al., 2000a,b). Similar to N-terminal inhibitors, novobiocin can promote client protein degradation and induce an HSR. However, systematic modification of the novobiocin pharmacophore has identified N-{7-[(2R,3R,4S,5R)-3,4-dihydroxy-5-methoxy-6,6-dimethyl-tetrahydro-2H-pyran-2-yl]oxy}-8-methyl-2-oxo-2H-chromen-3-yl}acetamide (KU-32) as a lead neuroprotective compound (Ansar et al., 2007; Lu et al., 2009) that exhibits at least a 500-fold divergence of Hsp70 induction from client protein degradation (Urban et al., 2010). Because Hsp70 is critical for the neuroprotective efficacy of KU-32 (see section III.D.3), this divergence provides an excellent therapeutic window to promote neuroprotection and minimize potential off-target toxicity in treating DPN.

2. Heat-Shock Protein Expression and DPN—An Impaired Defense Against Stress?? Chronic hyperglycemia imposes ischemic, hypoxic, oxidative, and apoptotic stress leading to widespread damage to proteins, cells, and tissues (Tomlinson and Gardiner, 2008; Obrosova, 2009b). Although the etiology of DPN is unrelated to one specific misfolded protein aggregate, hyperglycemia can promote the oxidative modification of amino acids (Obrosova et al., 2007a; Akude et al., 2010) that may impair protein folding (Muchowski and Wacker, 2005), decrease mitochondrial protein import (Baseler et al., 2011), and promote mitochondrial dysfunction (Fernyhough et al., 2010; Sivitz and Yorek, 2010; Chowdhury et al., 2011). Postmitotic neurons may be especially vulnerable to stress-induced protein denaturation, because diabetic nerves that exhibit neuropathological changes can have a lower expression of molecular chaperones. For example, 10 months of hyperglycemia markedly reduced Hsp70 levels in DRG from spontaneously diabetic Bio-breeding/Worcester rats, a model of type 1 diabetes. This correlated with an advanced neuropathy characterized by loss of neurotrophic components and a decrease in both myelinated/unmyelinated fibers (Kamiya et al., 2006). On the other hand, after only 4 months of diabetes in the Bio-breeding/Worcester rat model, Hsp27 and Hsp70 were increased in DRG (Kamiya et al., 2005). After only 1 month of STZ-induced diabetes, Hsp expression was unaltered or enhanced by heat shock depending on the tissue (Najemnikova et al., 2007). Thus, chaperone expression is differentially affected as the degeneration progresses. It is conceivable that the level of endogenous chaperones may be sufficient to counter early glycemic insults, but as diabetes becomes more prolonged, increased chaperone expression may represent a cytoprotective response that is eventually overwhelmed by the excessive metabolic disturbances associated with chronic diabetes. That being said, the question remains whether impaired Hsp70 expression is an initiator or
outcome of DPN. However, genetic knockout of the stress-inducible isoforms of Hsp70 (Hsp70.1 and Hsp70.3) caused no significant difference in the rate of onset or severity of a sensory hypoalgesia that developed in diabetic Hsp70 knockout mice (Urban et al., 2010). These data suggest that decreased Hsp70 expression is not an essential pathogenic component in DPN. Although there can be considerable functional redundancy between inducible (Hsp70) and constitutive (Hsc70) paralogs, we have noted that Hsc70 does not change in diabetic nerve, suggesting that its decline is also not a critical feature in the etiology of DPN. It is noteworthy that high anti-Hsp70 antibody levels, but not those of anti-Hsp60 antibody, were detected in an analysis of 531 patients with type 1 diabetes from the EURODIAB Study (Gruden et al., 2009). Independent of other risk factors and inflammation, a 50% lower likelihood of micro/macrovascular complications was associated with high anti-Hsp70 antibody levels (Gruden et al., 2009). Although the underlying origin of the antigen was not identified, these data suggest that elevated Hsp70 antibody levels may serve as a positive biomarker for patients less prone to developing diabetic complications (Gruden et al., 2009). It may be worthwhile to determine whether a relationship exists between Hsp70 antibody levels and the level of glyoxalase 1 expression (Jack et al., 2012) as a predictive biomarker pair for the development of DPN in humans (Rabbani and Thornalley, 2011).

3. 70-kDa Heat-Shock Protein and Neuroprotection in DPN. Although most other Hsps are abundantly distributed in the nerve, the inducible form of Hsp70 is weakly expressed, and its induction typically represents a cellular protective program adopted by neurons and glia during stress response (Pavlik et al., 2003; Pavlik and Aneja, 2007). Thus, physically or pharmacologically increasing Hsp expression may confer protection against neuropathic changes associated with diabetes. For example, patients with type 2 diabetes experienced reductions in blood glucose concentration and symptomatic neuropathy after receiving regular hot-tub treatment (Hooper, 1999, 2003). In diabetic rats, the HSF1 activators bimoclomol and arimoclomol (BRX-220) improved diabetic wound healing and nerve conduction deficits, respectively (Vigh et al., 1997; Kürthy et al., 2002); direct HSF1 activators may provide another pharmacologic route to modulating genes that express chaperones and antioxidant proteins (Neef et al., 2011). The potential effectiveness of α-lipoic acid in treating DPN has already been noted and α-lipoic acid therapy has been normalized. Hsp70 levels were decreased in a cohort of patients with type 1 diabetes and DPN (Strokov et al., 2000). Overexpression of Hsp70 in muscle is sufficient to improve insulin resistance and glucose utilization (Chung et al., 2008), and this metabolic correction would be anticipated to also improve DPN. However, the therapeutic benefits of Hsp70 induction in DPN do not seem to hinge on a metabolic correction and may be relatively nerve-specific (Urban et al., 2010, 2012b). On the other hand, modulating chaperones may improve the trophic effects of impaired insulin signaling in DPN, because several components of the insulin signaling cascade rely on interactions with heat shock proteins (Urban et al., 2012a).

We have been exploring the potential of a novel class of Hsp90 inhibitors as a pharmacologic tool to modulate Hsp70 in DPN. KU-32 is a C-terminal, novobiocin-based Hsp90 inhibitor (Fig. 2) that protected against neuronal cell death (Ansar et al., 2007). Despite being only a weak activator of the Hsp70 promoter compared with the prototypical N-terminal Hsp90 inhibitor geldanamycin (Fig. 3A), KU-32 increased Hsp70 expression in explant cultures of neonatal mouse sensory neurons (Fig. 3B) without affecting the expression of Hsc70 (Fig. 3C). It is noteworthy that weekly treatment of diabetic mice with KU-32 reversed a pre-existing mechanical and thermal hypoalgesia and improved deficits in both sensory and motor nerve conduction velocities (Urban et al., 2010). Moreover, after 16 weeks of diabetes in Swiss-Webster mice, intraepidermal nerve fiber density was decreased by 30%, and this loss of fibers was reversed by 10 weeks of KU-32 therapy (Urban et al., 2012b). Because no obvious improvement in plasma glucose or insulin levels were observed in mice administered KU-32, the protection did not require a metabolic correction of glucose utilization and probably resulted from a direct effect on chaperone expression in peripheral nerve. Indeed, despite the modest induction of Hsp70 induced by KU-32, it is central to drug efficacy. For example, KU-32 protected against neuregulin-induced demyelination in myelinated Schwann cell-sensory neuron cocultures prepared from wild-type mice (Figs. 4, A and B), but this effect was lost in neurons isolated from Hsp70 knockout mice (Figs. 4, C and D). Likewise, although KU-32 reversed multiple indices of DPN in wild-type mice, the drug was ineffective at reversing an insensate neuropathy in diabetic Hsp70 KO mice (Figs. 5, A and B) (Urban et al., 2010). These data agree with other reports supporting Hsp70 as a key neuroprotective chaperone preventing neuronal apoptosis (Bienemann et al., 2008) and improving neurodegenerative diseases associated with protein misfolding (Dickey et al., 2007; Luo et al., 2007). Thus, modulating Hsp70 may increase the tolerance of neurons and glia to the fluxing metabolic stresses associated with diabetes (Calcutt, 2010).

4. 70-kDa Heat-Shock Protein Family Members and Mitochondrial Function. Although it remains unclear how Hsp70 may antagonize the proneuropathic action of the established biochemical mediators of DPN, Hsp70 can improve oxidative stress and mitochondrial function. Hsp70 overexpression significantly potentiated the activities of glutathione peroxidase and glutathione reductase, leading to a higher GSH/GSSG ratio, attenuated ROS production, and improved cell survival under conditions of...
hypoxia and glucose deprivation (Guo et al., 2007). Likewise, overexpression of Hsp70 in astrocytes increased mitochondrial function and decreased neuronal injury after basal forebrain ischemia (Xu et al., 2010). Consistent with this result, KU-32 increased the translation of Hsp70, as well as other chaperones and MnSOD in hyperglycemically stressed sensory neurons. Increased expression of the molecular chaperones and MnSOD correlated with a decrease in mitochondrial superoxide levels and improved mitochondrial bioenergetics (Zhang et al., 2012). However, it remains to be determined whether chaperone induction improves mitochondrial bioenergetics to decrease oxidative stress or if enhanced expression of MnSOD and decreased superoxide production precedes improved respiratory function (Fig. 6). It is also worth noting that NADPH oxidases can serve as a potential source of superoxide in DPN (Coppey et al., 2003). Hence, overexpression of Hsp70 in glucose-deprived astrocytes also inhibited proton leak and ROS accumulation (Ouyang et al., 2006; Xu et al., 2010).

How Hsp70 may improve mitochondrial function is unresolved. Drp1 is a mitochondrial fission protein that may contribute to mitochondrial dysfunction in DPN (Vincent et al., 2010). A recent report indicates that inhibiting Drp1 translocation and phosphorylation in podocytes decreased oxidative stress and improved features of diabetic nephropathy (Wang et al., 2012). Given the ability of KU-32 to decrease mitochondrial superoxide levels in hyperglycemically stressed sensory neurons (Zhang et al., 2012), it will be important to determine whether this effect may be mediated via Hsp70-dependent interactions with Drp1 or upstream kinases in diabetic sensory neurons. Alternatively, oxidative modification of Drp1 leads to formation of tetramers and larger aggregates. Because modified Drp1 has enhanced GTPase activity, which can promote mitochondrial fission (Nakamura and Lipton, 2010), Hsp70 may decrease organelar fission by aiding Drp1 refolding. However, a folding-incompetent, ATPase-deficient Hsp70 mutant that maintained the ability to bind and aid the solubility of denatured proteins still improved mitochondrial function in cultured astrocytes (Ouyang et al., 2006). These data imply that the folding competency of Hsp70 may not be a key feature for protection. It is noteworthy that combining Hsp70 overexpression with inhibition of its ATPase activity by methylene blue increased the protea-
somal degradation of τ (Jinwal et al., 2009). Although mitochondrial dysfunction in DPN is not necessarily associated with a single protein aggregate, it will be interesting to determine whether increasing protein clearance by combining an Hsp70 inducer with a selective ATPase inhibitor may prove beneficial in treating DPN, as has been suggested for cancer therapy (Koren et al., 2010). Finally, cytosolic Hsp70 and Hsp90 interact with TOMM70, a mitochondrial outer membrane translocase, to mediate import of nuclear-encoded proteins (Young et al., 2003). Because 99% of the mitochondrial proteins arise from nuclear genes and need to be imported into the organelle (Schmidt et al., 2010), Hsp70 may facilitate the replacement of oxidatively damaged mitochondrial proteins (Fig. 6).

Cytosolic Hsp70 may also have help from other Hsp70 family members in decreasing oxidative stress. Genetic overexpression of the mitochondrial paralog of Hsp70 (mortalin/Grp75/mtHsp70) is sufficient to protect cultured primary astrocytes from ischemia-induced oxidative damage and death (Voloboueva et al., 2008). Likewise, mtHsp70 overexpression in brain reduced oxidative stress markers and improved mitochondrial function induced by ischemia/reperfusion (Xu et al., 2009). Overexpression of mtHsp70 in cardiac myocytes also potentiated the activity of respiratory chain complexes III and IV and abrogated ROS generation and lipid peroxidation after hypoxia/reoxygenation (Williamson et al., 2008). Using an unbiased proteomic screen, we recently identified that KU-32 also increased translation of MnSOD and mtHsp70 in hyperglycemic stressed neurons (Zhang et al., 2012). Unfortunately, it is unclear how KU-32 may increase mtHsp70 expression, because it is not induced as part of the classic heat-shock response (Deocaris et al., 2006). Nevertheless, approaches that can increase both cytosolic and mitochondrial Hsp70 family members are likely to improve mitochondrial function in DPN via effects on decreasing oxidative stress, aiding the refolding of oxidatively damaged and

![Fig. 4. Hsp70 is required for KU-32 to protect against neuregulin-induced demyelination. Myelinated mouse SC-DRG neuron cocultures were prepared from wild-type (A and B) or Hsp70 knockout (KO; C and D) mice and treated overnight with vehicle or 1 μM KU-32. The cultures were treated with PBS or 200 ng/ml neuregulin-1 for 4 days, and myelin segments were visualized by staining the cultures for myelin basic protein. Total cell number was assessed by staining nuclei with 4,6-diamidino-2-phenylindole. Cell Profiler (http://www.cellprofiler.org) was used to calculate the total myelin segment area from six fields per six coverslips per treatment. The results are expressed as a fold of the untreated control and are an average of three experiments per genotype. *p < 0.05 versus KU-32, †p < 0.05 versus NRG. Arrows, examples of myelin internodes. [Modified from Urban MJ, Li C, Yu C, Lu Y, Krise JM, McIntosh MP, Rajewski RA, Blagg BS, and Dobrowsky RT (2010) Inhibiting heat-shock protein 90 reverses sensory hypoalgesia in diabetic mice. ASN Neuro 2:e00040. Open Access from Portland Press Limited and the American Society for Neurochemistry.]
Exercise has long been recognized as a part of the therapy for the management of diabetes. However, 31% of patients with type 2 diabetes fail to participate in basic physical activity, possibly because of secondary diabetic complications such as DPN (Nelson et al., 2002). Recent studies have shown that exercise has beneficial effects on painful symptoms in the setting of diabetes. In patients with type 1 or 2 diabetes who did not display any signs or symptoms of DPN, aerobic exercise (brisk walking on a treadmill) reduced the onset of both motor (0 versus 17%) and sensory (7 versus 30%) neuropathy compared with patients with diabetes who were sedentary (Balducci et al., 2006). As for patients with impaired glucose tolerance, lifestyle interventions (diet and exercise counseling) can also improve painful symptoms (Smith et al., 2006). The efficacy of exercise in exerting a similar effect in patients with diabetes who have signs and symptoms of DPN has yielded similar results. Patients with DPN who exercise regularly experience significant improvements in electrophysiological deficits and neuropathic symptoms, which is accompanied with increases in intraepidermal nerve fiber branching (Fisher et al., 2007; Hung et al., 2009; Kluding et al., 2012). In diabetic animals, exercise prevents myelin damage and attenuates changes in voltage-gated Ca2+ channel function, thereby ameliorating electrophysiological deficits that occur in DPN (Selagzi et al., 2008; Shankarappa et al., 2011). In addition, exercise can increase expression of Hsp70 (Paulsen et al., 2007; Ogata et al., 2009). Exercise leads to long-term activation of muscle fibers, which increases temperature, metabolic disturbances, and oxidative stress, thus activating a putative heat-shock response (Fig. 2) (Noble et al., 2008). In a transgenic mouse model of Alzheimer’s disease, exercise repressed neuronal cell death, which correlated with an up-regulation of Hsp70 expression in the mice that exercised (Um et al., 2011). Likewise, aerobic exercise increased Hsp70 expression in diabetic rats, although the magnitude of this increase was less than that observed in nondiabetic animals (Atalay et al., 2004). These studies strongly suggest that exercise has beneficial actions on alleviating pain in patients with diabetes and provide a potential mechanism by which exercise beneficially maintains neural pain circuitry. It will be insightful to use the Hsp70 knockout mice to examine the contribution of Hsp70 in mediating the benefits of exercise in alleviating aspects of DPN.

It is tempting to speculate that exercise coupled with pharmacologic modulation of chaperone signaling may offer an alternative measure toward managing either painful or insensate DPN, assuming that patients are able to adequately exercise without risk. However, although exercise may be a cost-effective treatment, it may exacerbate complications or have unintended consequences because of pre-existing diabetic complications (Chipkin et al., 2001). Before starting an exercise program, patients with diabetes should be evaluated to minimize the risk of exacerbating existing macro- and microvascular complications. Patients with diabetes who experience peripheral neuropathy should consider only exercises they can tolerate without intensifying their painful symptoms. Most importantly, all patients should monitor blood glucose levels before, during, and after all bouts of exercise. For patients with diabetes, the overall benefits of exercise in preventing and reducing painful DPN are clearly significant (Balducci et al., 2006; Smith et al., 2006). Therefore, clinicians and patients must work together to maximize exercise-induced benefits while minimizing the adverse events to obtain a healthier lifestyle and improve overall quality of life (Chipkin et al., 2001).
Fig. 6. Potential mechanisms by which modulating chaperones may increase mitochondrial function in DPN. Diabetes-induced increases in mitochondrial ROS may promote formation of oxidatively modified proteins, which contribute to a decreased respiratory capacity. Increasing transport of proteins to mitochondria may aid in replacing damaged proteins. Hsp70 is involved in protein transport to mitochondria and contributes to the internalization of multispansing inner mitochondrial membrane proteins via TOM70. Although not required, Hsp70 may also facilitate the transfer of preproteins containing a mitochondrial targeting sequence, such as MnSOD, to TOM22. mtHsp70 is a requisite chaperone for internalization of preproteins before final proteolytic processing. Within the organelle, Hsp60 and mtHsp70 may aid the refolding of oxidatively damaged mitochondrial proteins. In the presence of sufficient levels of NADPH, Hsp70 may also increase the GSH/GSSG ratio in the cytoplasm by increasing the activity of glutathione peroxidase and reductase. TOM and TIM, translocases of the outer and inner mitochondrial membrane, respectively; Pi, inorganic phosphate; CoQ, coenzyme Q.

IV. Conclusions

It is clear that oxidative stress and mitochondrial dysfunction contribute to the development and progression of DPN. The complex and inter-related mechanisms that lead to these pivotal deficits render it unlikely that the effective targeting of any one pathogenic pathway will offer comprehensive efficacy against DPN. This difficulty is further compounded by the fact that not all pathways may contribute to neuropathy in a temporally and/or biochemically uniform fashion, given the prolonged natural history of disease development (decades), nutritional influences, and genetic and phenotypic heterogeneity in humans. Although the idea of pharmacologically targeting multiple pathogenic pathways that contribute to DPN is not new, adding the modulation of endogenous, cytoprotective molecular chaperones to the mix is a novel supplement that does not rely on inhibiting any one particular mechanism. However, activating cytoprotective pathways is not necessarily a panacea and may succumb to similar limitations that have undermined the successful development of therapeutics that target causal mechanisms. Given that there are multiple pathogenic pathways, a logical and likely inversion is that multiple pathways also regulate neuronal recovery from and tolerance to recurrent glycemic damage. If cytoprotective pathways show a similar variability in efficacy between patients and over time, or poorly translate from the animal models, then emphasizing any one of these mechanisms may not yield a better outcome than our past emphasis on targeting individual pathogenic features of DPN. Although a valid concern, the bleak landscape of therapeutics for DPN would seem to necessitate broadening our paradigms toward disease treatment. It will no doubt remain important to always emphasize good glycemic control and to promote some level of adequate exercise. However, pharmacologic approaches that converge on decreasing inflammation, improving oxidative stress, and stimulating mitochondrial bioenergetics are likely to prove beneficial to the long-term management of DPN. This goal may be realized by targeting causation to limit glycemic damage while also enhancing chaperones to tolerate chronic diabetic stress.

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