

Novel pathogenic pathways in diabetic neuropathy

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Diabetic peripheral neuropathy (DPN) is a common complication affecting more than one third of diabetes mellitus (DM) patients. Although all cellular components participating in peripheral nerve function are exposed to and affected by the metabolic consequences of DM, nodal regions, areas of intense interactions between Schwann cells and axons, may be particularly sensitive to DM-induced alterations. Nodes are enriched in insulin receptors, glucose transporters, Na⁺ and K⁺ channels, and mitochondria, all implicated in the development and progression of DPN. Latest results particularly reinforce the idea that changes in ion-channel function and energy metabolism, both of which depend on axon–glia cross-talk, are among the important contributors to DPN. These insights provide a basis for new therapeutic approaches aimed at delaying or reversing DPN.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia and dysinsulinemia, often accompanied by a more complex metabolic syndrome (see Glossary). Currently, DM affects more than 366 million people worldwide (<http://www.idf.org/diabetesatlas/5e/>) and this number is rapidly increasing. Two major types of DM are recognized: type 1 DM (T1DM) and type 2 DM (T2DM). Patients affected by either condition may develop severe chronic complications including retinopathy, nephropathy, neuropathy, and cardiovascular disease [1]. Diabetic peripheral neuropathy (DPN) is one of the most common complications of DM with an estimated prevalence ranging, in relation to the degree and duration of the disease, from 13% to 58% of DM patients [2,3]. DPN affects both sensorimotor and autonomic parts of the peripheral neural system (PNS). The most common clinically recognized form is diabetic sensorimotor polyneuropathy (DSPN) [4]. Its earliest pathological symptoms are detectable in feet and hands and are predominantly characterized by structural defects of axons (axonopathy) and Schwann cells (schwannopathy). The diversity and variable presence of both ‘positive’ and/or ‘negative’ symptoms underscores the complexity of this disease (Box 1) [2,3]. Substantial insight into the underlying pathophysiological

mechanisms was obtained through the characterization of the available rodent models of DM (Box 2, Table 1). DSPN represents a major health problem because it may present with excruciating neuropathic pain and is responsible for substantial morbidity, increased mortality, and impaired quality of life [5]. In addition, it was recently suggested that the prevalence of DSPN is increased even in prediabetes, in particular if impaired glucose tolerance is combined with impaired fasting-glucose [6].

Glossary

Action potential (AP): a change in the resting membrane potential of excitable cells due to the successive opening of voltage-gated Na_v and K_v channels. It consists of three phases: de-, re-, and hyperpolarization.

Cellular respiration: a process producing ATP which can be divided into three steps: cytosolic glycolysis, the mitochondrial citric acid cycle, and electron transport chain/oxidative phosphorylation.

Hexosamine pathway: glucose is metabolized to amino sugars, for example UDP-N-acetylglucosamine, via the hexosamine pathway. This pathway is particularly increased during hyperglycemia.

Insulin resistance: the inability of peripheral tissues (e.g., muscle, adipocytes, liver, and peripheral nerves as outlined here) to respond to circulating insulin.

Juxtaparanode: region under the myelin sheath adjacent to the paranode; highly enriched in K_v1 channels which are important for AP repolarization and nerve excitability.

MCT: monocarboxylate transporters, present in the plasma membrane and mitochondria of both glial and neurons, are involved in the transportation of pyruvate and lactate.

Metabolic syndrome: a group of metabolic disorders, including predominantly obesity, hyperglycemia, dysinsulinemia, dyslipidemia, and hypertension, that raise the risk for cardiovascular diseases and DM.

Methylglyoxal: methylglyoxal is a byproduct of glycolysis that is highly enriched under DM. It promotes the formation of AGEs and is suggested to impair mitochondrial function.

Na⁺/K⁺-ATPase: ATP-dependent ion pump maintaining resting membrane potential by extruding three Na⁺ ions against two K⁺ ions for each ATP molecule consumed. This represents one of the most energy-demanding subcellular processes.

Nodal domains: the generic term to describe distinct domains between two internodal segments of Schwann cell myelin, composed of the node of Ranvier, paranode, and juxtaparanode.

Node of Ranvier: unmyelinated gap of ~1 μm between two myelin segments of peripheral nerves that is covered by Schwann cell microvilli. An area of AP generation due to a high accumulation of Na_v channels. It also contains K_v channels.

Oxidative stress: situation in a cell if there are more oxidants (e.g., superoxide, peroxynitrite) than antioxidants, potentially leading to cell damage.

Paranode: region between the node of Ranvier and juxtaparanode characterized by close axon–glial interactions. Schwann cell paranodal loops serve as a diffusion barrier between internode and node.

Type 1 diabetes mellitus (T1DM): inability to produce adequate amounts of insulin as a consequence of an autoimmune reaction against pancreatic β cells (affects ~10% of DM patients).

Type 2 diabetes mellitus (T2DM): the most common form of DM (affects ~90% of DM patients) initially caused by a defective response of target tissues to secreted insulin.

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Keywords: diabetic neuropathy; insulin resistance; axon; Schwann cell; mitochondria; ion channels.

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Box 1. Clinical pattern of DSPN

The most frequent phenotype of DPN is symmetrical damage of peripheral nerves progressing from the distal to more proximal sites. This form is termed diabetic sensorimotor polyneuropathy (DSPN) and its clinical features are summarized here. The symptoms of less-common forms of DPN were previously covered by others [2–4]. The early phase of DSPN is characterized by functional defects that might be reversible. Typical symptoms can be classified into positive sensory symptoms (paresthesia, allodynia, hyperalgesia, and pain), negative sensory symptoms (injury insensitivity potentially leading to foot ulcers, numbness) and less-prominent motor symptoms (muscle atrophy, weakness, loss of reflexes). In addition, functional impairment of peripheral nerves, determined by a reduction of the sensory and/or motor nerve conduction velocity (SNCV and MNCV, respectively), is often present [2–4]. With duration of the disease, irreversible structural defects of the peripheral nerves are observed, leading to progressive worsening of the above-mentioned symptoms. Neurons, Schwann cells, and endothelial cells are affected but the specific contribution of these cell types and the order of events leading to the DSPN phenotype is still unclear [2,13]. The presence of axonal loss in diabetic patients led to the suggestion that DSPN is primarily an axonopathy. However, it is also possible that axonal loss may be a secondary consequence of a disruption of its appropriate Schwann cell support. Corneal and intraepidermal nerve fiber countings are two emerging screening methods that may help to detect early loss of unmyelinated C-fibers and thin myelinated A δ -fibers in diabetic patients. Subsequent loss of larger myelinated fibers can be determined by a reduction in compound muscle action potential (CMAP) amplitude. Nerve biopsies often present axonal loss accompanied by signs of neuroregeneration, which however mostly fails [2]. In addition, biopsies of T1DM patients show early axonal swellings followed by axonal and paranodal degeneration, which is less prominent in T2DM patients. Further differences between the DSPN phenotype in T1DM and T2DM were described previously [13]. The exact cause of the early reduction in NCV is currently unknown. Demyelination and/or segmental demyelination contributes to the reduction in NCV associated with temporal dispersion and prolonged CMAP latency, especially at later stages of the disease [2,13]. Although demyelination is so far considered to be a consequence of axonal loss, a possible primary role of Schwann cells in the pathology of DSPN, especially through their role in axonal metabolic support, has begun to emerge.

In this review we focus on the diabetes-induced mechanisms underlying pathophysiological changes in neurons, their axons, and in Schwann cells. In addition to neurons/axons and glia, the PNS contains endothelial capillaries, residual macrophages and fibroblasts (Figure 1), which also play a role in DSPN [7,8] but will not be covered in this review. In particular, we concentrate on the crosstalk between axons and Schwann cells, which is most prominent at nodal domains of peripheral nerves, and we highlight the importance of ion-channel physiology and energy metabolism in alterations of PNS function under diabetic conditions. Finally, we evaluate the potential of these discoveries for the development of new therapeutic approaches aimed at delaying or preventing DSPN symptoms.

PNS: structural and functional considerations

The increased vulnerability of the PNS under diabetic conditions relative to other tissues might result from its structure, function, and/or particular metabolic needs. The principal function of peripheral nerves is to transmit electrical signals, termed action potentials (AP), between the periphery and the central nervous system (CNS). This process is executed by small unmyelinated and large

myelinated fibers, which both can extend over 1 m in length. Myelin is a lipid-rich membrane [9] produced by Schwann cells in the PNS that electrically isolates axons with a diameter larger than $\sim 1 \mu\text{m}$. Tightly controlled interactions between axons and Schwann cells regulate both the process of myelination [10] and the formation of the nodal domains [11]. These nodal domains are organized in three distinct areas: the nodes of Ranvier, flanked by paranodes and juxtaparanodes. This unique structural organization allows a high enrichment of voltage-gated Na $^+$ and K $^+$ channels at the nodes of Ranvier (Na $_v$ 1.6, K $_v$ 7, and K $_v$ 3, respectively) and at juxtaparanodes (K $_v$ 1) that are defined by paranodal junctions which are composed of barrier-like adhesion complexes [11] (Figure 1). Together with the presence of optimized amounts of myelin, the nodal structure is responsible for fast, saltatory AP conduction. Nodal degeneration, axon–glial dysjunction, and altered ion-channel localization have been associated with both human and experimental DSPN [12,13]. In addition to the above-mentioned Na $_v$ and K $_v$ channels, nodal regions are also enriched in the presence of other players involved in DSPN: insulin receptors and glucose transporters (Figure 1). Importantly, myelinating glial cells were recently identified as local suppliers of glucose-derived energy for underlying axons thus revealing another form of axon–glia interactions [14–16] that may also play a role in DSPN.

Insulin signaling and glucose/lactate metabolism in the PNS*Insulin and insulin receptor*

Both T1DM and T2DM lead to defects in insulin signaling in the PNS that contribute to the DSPN phenotype [17]. Insulin signaling requires interaction of insulin with the insulin receptor (IR), which leads to IR autophosphorylation on intracellular tyrosine residues and subsequent activation of a complex network of intracellular signaling pathways [17]. IR is expressed in neurons and glia of both the CNS and PNS. Importantly, nodal regions of axonal membranes and Schwann cells are enriched in IRs [18–21] (Figure 1). In neurons, IR was demonstrated to be expressed on mitochondria, and insulin treatment prevented hyperglycemia-induced mitochondrial depolarization [2]. Moreover, near-sciatic nerve or intrathecal insulin application partially reversed some of the DPN phenotypes in rodents [2].

Insulin, together with other growth factors, plays a crucial role in neurotrophic support and neuronal regeneration of injured axons, both of which are affected by diabetic conditions [2,22]. Recent data also revealed that chronic insulin stimulation (a model of T2DM hyperinsulinemia) leads to a decreased capacity of dorsal root ganglion (DRG) neurons in culture to respond to acute insulin stimulation [21,23]. Part of this insulin resistance in DRG neurons is mediated by their reduced sensitivity to insulin-induced Akt activation. These data suggest that neurons develop insulin resistance similarly to classical ‘insulin-sensitive’ tissues. As a consequence of their reduced responsiveness to the neurotrophic properties of insulin under hyperinsulinemia, neurons may have impaired regenerative potential in the situation of neuronal injury

Box 2. Modeling DSPN in rodents

Clinical studies have led to substantial insight into the symptoms of DSPN, its patterns of neurological involvement, and its pathological consequences in DM patients [4]. Although these data are crucial for the scoring/classification of DSPN in patients which is necessary for epidemiological surveys and clinical trials, they provide only partial insight into the pathophysiological mechanisms underlying DSPN. To overcome these limitations, substantial effort was put into the generation and characterization of animal models of DSPN. Although other models were previously characterized (e.g., diabetic dogs and cats), the majority of recent data were generated through characterization of diabetic rodents [12,13,32,36,94,102–104] (Table 1). Their molecular characterization led to the discovery of numerous pathways affected by diabetic conditions [37] and they play a critical role in evaluation of therapeutic approaches aiming at improvement of diabetic phenotypes. However, variation in structural defects and therapeutic benefits between rodent models and patients, probably as a consequence of differences in lifespan (and therefore duration of DM), nerve length, and/or metabolic needs, indicates potential limitations of rodents as models of DSPN.

and/or neuropathy [21,23]. In light of their above-mentioned role in axonal, support it would be relevant to investigate if Schwann cells also develop insulin resistance under diabetic conditions.

In addition to its neurotrophic properties, it was observed in cultured Schwann cells that insulin promotes the expression of myelin protein zero (MPZ), a commonly used marker of myelin gene expression [19]. Furthermore, the endoneurial expression of the transcription factor SREBP-1c (one of the key regulators of the expression of genes involved in fatty acid metabolism) is decreased in a rat model of T1DM, and SREBP-1c expression is induced in primary cultured Schwann cells treated by insulin [24]. However, further studies will be needed to clarify how the above-mentioned regulatory role of insulin on glial cells contributes to their pathological alterations under diabetic conditions.

Glucose and lactate transporters

Glucose uptake and utilization are not insulin-dependent in the PNS [25]. Neurons and Schwann cells import glucose by a process of facilitated diffusion mediated by members of the GLUT (glucose transporter) family of membrane transport proteins [26]. The major neuronal type is GLUT3, but other forms including GLUT1, GLUT4, and GLUT8 are also present [27,28]. Although these GLUTs are present in both soma and axons/dendrites in the PNS, GLUT3 seems to be enriched in the axonal nodal regions (Figure 1) [29]. Schwann cells predominantly express GLUT1 and GLUT3 [29], both of which localize principally in the nodal areas of myelinated fibers.

Concerning the potential involvement of GLUTs in DSPN pathophysiology, transcriptional studies failed to detect any significant expression changes induced by either T1DM or T2DM in glial or neuronal cells [30–32]. However, it is possible that the diabetic condition affects GLUT localization, as previously observed for GLUT4 in cultured cerebellar neurons [33], or leads to post-translational GLUT changes potentially altering its function in neurons and/or glial cells.

Lactate, which is generated from glucose through glycolysis, has also been proposed as a crucial energy source in the nervous system [15]. Lactate is transported by monocarboxylate transporters (MCTs). In the CNS, myelinated glia express MCT1 whereas axons express MCT2 [14,15], and it was shown that, in cultured neurons, MCT2 subcellular localization is in part regulated by insulin [34]. Although perineurial cells of the PNS also express MCT1 [35], its detailed endoneurial expression has not yet been explored.

Mechanisms implicated in DSPN

Molecular pathways behind endoneurial glucose-induced neuroglial toxicity

In addition to the above-mentioned changes in insulin signaling, ongoing hyperglycemia is recognized as the main contributor to the development of DSPN in T1DM and T2DM [3], and leads to defects in multiple molecular pathways [2,36,37]. Part of the excessive intracellular glucose is metabolized through the polyol pathway, leading to its reduction to sorbitol by aldose reductase (AR) and further oxidation to fructose by sorbitol dehydrogenase. The polyol pathway was the first route suggested to link DM hyperglycemia to DSPN [25]. In the PNS, AR is predominantly localized in Schwann cells, suggesting that the polyol pathway may predominantly contribute to the schwannopathy-related phenotype of DSPN (Figure 2) [2,25]. Interestingly, AR inhibitors, which should mostly act in Schwann cells, were shown to correct some of the axonopathy- and schwannopathy-related phenotypes present in rodent DSPN models [36]. Unfortunately, the effect of these drugs was less clear in clinical trials [5]. The accumulation of sorbitol and fructose contributes to depletion of myo-inositol and taurin, Na⁺/K⁺-ATPase inhibition, intracellular Na⁺ accumulation, axonal swelling, axon-glia dysjunction, and reduced nerve conduction velocity (NCV). Excessive glucose also leads to: (i) the formation of advanced glycation end-products (AGEs) that results from the non-enzymatic glycosylation of proteins, nucleotides, or lipids; (ii) the aberrant activation of protein kinase C (PKC); (iii) the glucose shunt through the hexosamine pathway; as well as (iv) the formation of oxidative and nitrosative stress associated with an impaired anti-oxidative defense. Interestingly, nitrosative and, in particular, oxidative stress is a consequence of most described pathways [36]. Peroxynitrite, a highly reactive oxidant, is a product of the reaction of superoxide (O²⁻) with nitric oxide (NO). Among its other actions, peroxynitrite activates poly(ADP-ribose) polymerase (PARP), whose overexpression may lead to energy depletion and apoptosis.

Mitochondrial dysfunction related to diabetic conditions

Although the exact contribution of various pathways affected by hyperglycemia (together with changes in insulin signaling, impaired neurotrophic support, and dyslipidemia) to the DSPN phenotype is still debated, many directly or indirectly affect glial and axonal mitochondria [3,37,38] (Figure 2). To fulfill their functions, neurons and their axons require a high amount of energy, predominantly produced through mitochondrial oxidative phosphorylation [39]. In axons, a specific accumulation of mitochondria

Table 1. Rodent models of DSPN^{a,b}

| Model | Type of DM | NCV alterations | Thermal and mechanical sensitivity | Axonal degeneration | Myelin defects | Refs |
|--------------|------------|---------------------------|---|--|--------------------------------|-------------|
| Mice | | | | | | |
| STZ-induced | T1DM | Reduced SNCV and MNCV | Variable defects in thermal and mechanical sensitivity dependent on dose of STZ | Reduced number of IENF, axonal loss | Hypomyelination | [2,102] |
| Akita | T1DM | Reduced SNCV and MNCV | Thermal hypoalgesia | Normal IENF, no axonal loss | No demyelination detected | [32,102] |
| NOD | T1DM | Reduced SNCV and MNCV | Thermal hypoalgesia | Reduced number of IENF, axonal loss | No changes in myelin thickness | [103] |
| <i>db/db</i> | T2DM | Reduced SNCV and MNCV | Thermal hypoalgesia, mechanical hyperalgesia | Reduced number of IENF, axonal atrophy | No demyelination detected | [12,30,102] |
| <i>ob/ob</i> | T2DM | Reduced SNCV and MNCV | Thermal hypoalgesia, mechanical allodynia | Reduced number of IENF | Not yet characterized | [102] |
| Rats | | | | | | |
| STZ-induced | T1DM | Reduced MNCV and SNCV | Thermal hyperalgesia passing over to hypoalgesia, mechanical allodynia | Axonal loss | Demyelination | [36,104] |
| BB/Wor | T1DM | Reduced MNCV and SNCV | Thermal hyperalgesia | Axonal loss, nodal degeneration | Mild demyelination | [13] |
| ZDF | T2DM | Reduced SNCV and MNCV | Thermal hypoalgesia, mechanical hypoalgesia, tactile allodynia | Loss of IENF | Mild hypomyelination | [94] |
| BBZDR/Wor | T2DM | Reduced MNCV, normal SNCV | Thermal hyperalgesia | Mild axonal loss | Demyelination | [13] |

^aA summary of clinically related phenotypes for each model is presented. It is important to note that, among other parameters, diabetes duration, animal gender, genetic background, and diet may affect the onset and robustness of various alterations.

^bAbbreviations: BB/Wor, BioBreeding/Worcester; BBZDR/Wor, BioBreeding Zucker diabetic rat/Worcester; *db/db*, diabetic (*Lepr* mutant); DM, diabetes mellitus; IENF, intraepidermal nerve fibers; MNCV, motor nerve conduction velocity; NCV, nerve conduction velocity; NOD, non-obese diabetic; *ob/ob*, obese (*Lep* mutant); SNCV, sensory nerve conduction velocity; STZ, streptozotocin; T1DM/T2DM, type 1/type 2 diabetes mellitus; ZDF, Zucker diabetic fatty.

is present in regions of high metabolic activity, including nodal domains, axonal initial segments, and distally at nerve termini [40] (Figure 1). Importantly, recent data indicate that axons in both the CNS and PNS depend on the energy metabolism of glial cells to sustain their energy demands and function [14–16]. Similarly to the DSPN phenotype, it was shown that disrupted Schwann cell mitochondrial function in connection with perturbed glial support causes primary neuronal degeneration first in the unmyelinated fibers and subsequently in myelinated fibers [16]. In the PNS, the majority of glucose is taken up by Schwann cells [41], and it was proposed that in Schwann cells, glucose is preferentially metabolized via glycolysis, a process of low energy-gain [39]. It is possible that energetic metabolites derived from this process, such as lactate, or alternatively from Schwann cell lipid metabolism-related metabolites [24,42], are transported from glia to the axon where they are oxidized to produce ATP. As discussed above, the localization of such transporters (GLUTs, MCTs) might be affected by the diabetic condition. Moreover, both lactate and pyruvate concentrations are affected under diabetic conditions, suggesting that Schwann cell capacity to support the underlying axons may indeed be disturbed under DM [43,44] (Figure 2).

Mitochondrial gene or protein expression and function under diabetic conditions was recently analyzed in various studies in both the glial (sciatic or sural nerve) or neuronal (DRG) compartments. These data revealed that there is a short-term increase in mitochondrial gene or protein expression after <1 week of DM [45,46]. However, this increase was not found after medium-term DM exposure

(between 1 week and 3 months of DM) [32,45–47], and was even found to be converted to a significant decrease in mitochondrial gene and protein expression in chronic DM models (>3 months of DM) [30,31,47,48]. In contradiction to the above-mentioned expression data, the ATP production of Schwann cells was diminished from the earliest DM stages without detectable production of mitochondrial oxidative stress [46]. The decrease in mitochondrial function was confirmed at later DM stages in DRG neurons, and was accompanied by reduced intramitochondrial oxidative stress [47,48]. These data indicate that reduced activity of neuronal and glial mitochondria does not necessarily correlate with changes in mitochondrial RNA or protein expression and is not associated with an increase in mitochondrial oxidative stress. Part of the reduced mitochondrial activity may be consequent upon the activation of alternative glucose-metabolism pathways that strongly limit mitochondrial supply of pyruvate and other substrates for mitochondrial respiration. More recently, peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), a key regulator of mitochondrial activity [49], was also shown to be downregulated in DRGs of diabetic animals [38]. Moreover, reactive oxygen species (ROS), which are produced in the cytosol (probably by pathways other than mitochondrial overactivation; e.g., the hexosamine or PKC pathways) (Figure 2), together with deficiency in insulin, might also contribute to decreased mitochondrial functionality under diabetic conditions [47,48,50]. Interestingly, changes in mitochondrial dynamics, which may affect their function, were also suggested to contribute to DSPN [51,52]. Similar mechanisms

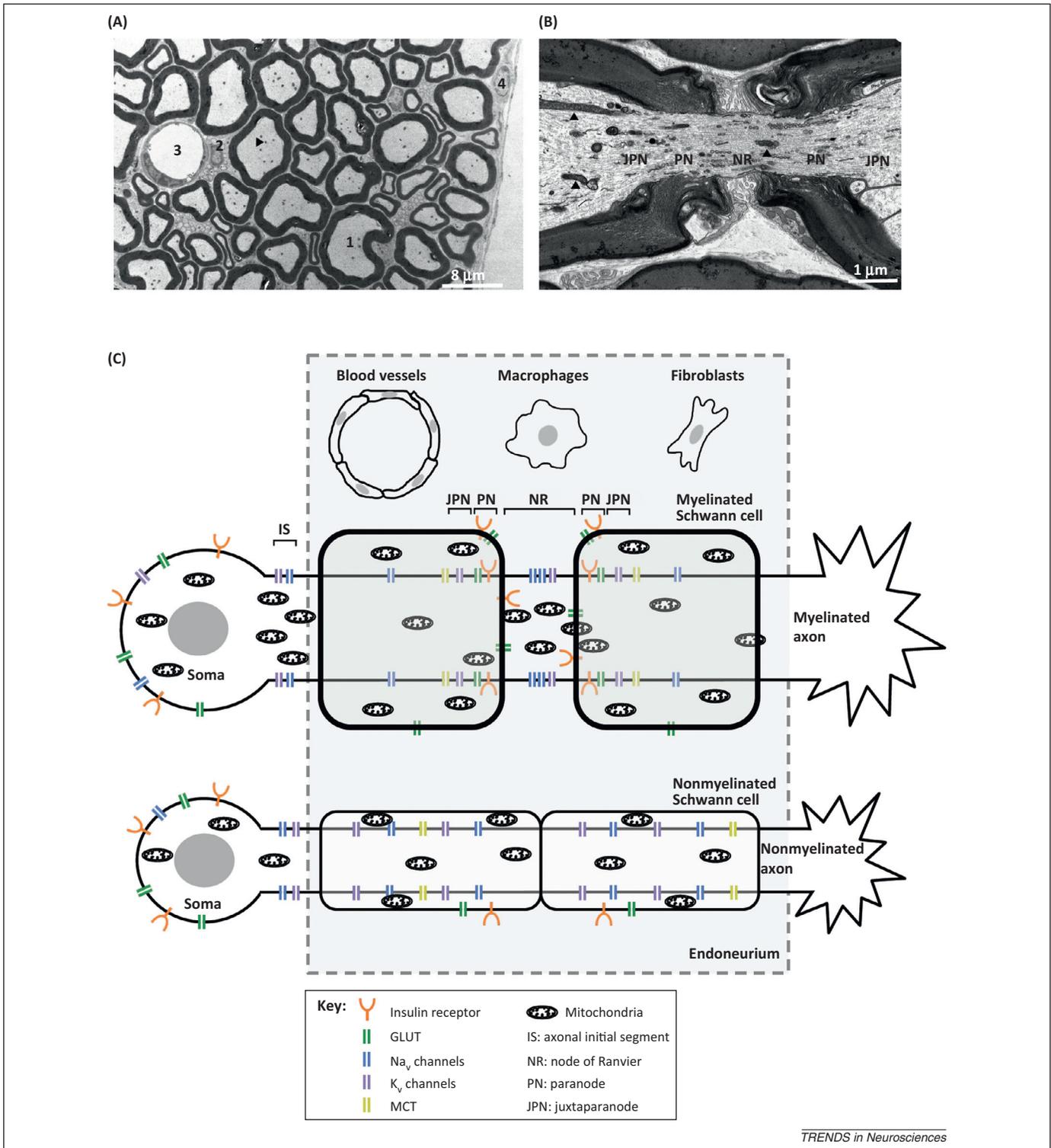


Figure 1. Structure of a peripheral nerve indicating components involved in the pathophysiology of diabetic sensorimotor polyneuropathy (DSPN). **(A)** Peripheral nerves (as shown by electron micrograph of a cross-section through adult mouse sciatic nerve) are composed of myelinated and/or unmyelinated fibers. In the peripheral neural system (PNS) endoneurium, myelinated fibers (1) are wrapped by individual Schwann cells at a 1:1 ratio, whereas each unmyelinated Schwann cell engulfs multiple axons in Remak bundles (2). Dense structures present in large axons are mitochondria (arrowhead). The endoneurium also harbors capillaries (3), fibroblasts (4), and macrophages (not present in this Figure). **(B)** Longitudinal section through the node of Ranvier (NR) also showing adjacent paranodes (PN) and juxtaranodes (JPN). Examples of mitochondria are shown by arrowheads. **(C)** Simplified representation of PNS structure. Insulin receptors (IR), glucose transporters (GLUT), mitochondria, and ion channels are widely distributed along unmyelinated fibers but accumulate at nodal domains of myelinated fibers. IR and GLUTs (GLUT1 and 3 for Schwann cells and GLUT1, 3, 4, and 8 for axons) are enriched at both Schwann cell and axonal membranes at NR and at PN. Na_v channels, in particular Na_v1.6, are accumulated at the NR, and K_v channels K_v1.1 and K_v1.2 at the JPN. Although distributed along the whole length of the myelinated axon, mitochondria are predominantly clustered at the NR and in the region of axonal initial segment (IS), which also has a particular channel composition (presence of Na_v1.2 channels). The neuronal cell bodies also express IR and GLUTs, similarly to the axonal compartment, but have a much broader ion-channel diversity. The position of the monocarboxylate transporters (MCT) is approximate because their expression in the PNS needs to be further characterized. Other constituents of the PNS endoneurium, including blood vessels, macrophages, and fibroblasts, which also potentially contribute to the DSPN phenotype, are not depicted in detail.

have been proposed to play a role in other neurodegenerative diseases including Alzheimer's disease, HIV, Friedreich ataxia, and Charcot–Marie–Tooth neuropathy type 2 [38].

Even a small reduction in mitochondrial function, and subsequent ATP depletion, may have considerable consequences for PNS function. Nerve conduction, axonal transport, and outgrowth following nerve injury are primarily ATP-dependent processes. All these processes involve predominantly the more distal part of axons, the region that is primarily affected by DSPN. ATP depletion may lead to reduced Na^+/K^+ ATPase activity that was previously suggested to contribute to the DSPN phenotype [53]. As a consequence, the axoplasmic Na^+ concentration may increase, which activates the $\text{Na}^+/\text{Ca}^{2+}$ pump and leads to an increase in the intracellular Ca^{2+} concentration, a common feature of DM [54]. The altered ion gradient will lead to reduced axonal membrane potential, opening of Na_v channels, and subsequent increase in axonal excitability as observed in DSPN [12,55–57]. The increase in intracellular Ca^{2+} may also contribute to the depolarization of the mitochondrial membrane [48,54] (Figure 2). Finally, the progressive rise in axonal Ca^{2+} may initiate calpain activation that is known to contribute to axonal degeneration [58].

Ion conductance under diabetic conditions

Axonal AP transmission depends not only on energy supply but also on a precise interplay of Na^+ and K^+ ion currents, regulated by the ATP-dependent Na^+/K^+ -ATPase and voltage-gated ion channels. Altered ion-channel expression, distribution, and function contribute to the phenotype of peripheral neuropathies, including DSPN, and to diabetic neuropathic pain [3,11–13]. Pain pathogenesis in DSPN is highly complex, affecting both the PNS and CNS (e.g., changes in expression and/or function of ion channels in peripheral nerves, DRGs, and spinal dorsal horn), and is still not well understood. A detailed description of painful DSPN can be found elsewhere [3,59,60]. Here we will concentrate on the two main players involved in AP conduction in the PNS axons – the Na_v and K_v channels. In myelinated fibers, they are enriched at the node of Ranvier and in juxtaparanodal areas, in close proximity to GLUTs, IR, and mitochondria (Figure 1), and this localization is dependent on proper axon–glia interaction [11].

Na_v channels

Of nine different Na_v channel subtypes ($\text{Na}_v1.1$ to $\text{Na}_v1.9$), the expression of $\text{Na}_v1.1$, 1.2, and 1.6 to 1.9 was previously detected in the adult PNS. Large DRG neurons predominantly express $\text{Na}_v1.1$, 1.6, and 1.7 in their soma, whereas the expression of $\text{Na}_v1.8$ and 1.9 under physiological conditions is restricted mainly to small DRG neurons [61].

A general increase in Na_v current in DRG neurons has been described to contribute to peripheral nerve hyperexcitability (PNH) in T1DM models [57,62,63], most likely underlying the pain phenotype in DSPN. However, the neuronal expression of Na_v subtypes is variable between different studies [62–66]. Na_v channel expression is in part regulated by neurotrophic support [67]. Reduced neurotrophic support [e.g., nerve growth factor (NGF), brain-derived neurotrophic factor, glial cell-derived neurotrophic factor signaling], which

may be a consequence of distal axonal degeneration under diabetic conditions, was previously suggested as a link between DM and PNH [64,66], and could partially explain reduced Na_v channel expression in DM.

Most studies on Na_v channel expression and function in sensory neurons used DRG-derived neuronal somas as a model. However, Na_v channel distribution and physiology differ between soma, axons, and axonal termini. $\text{Na}_v1.6$ is the major Na_v channel at the node of Ranvier of myelinated axons [11], $\text{Na}_v1.7$ along unmyelinated axons, and $\text{Na}_v1.2$ at the axonal termini [68] (Figure 1). Importantly, precise nodal localization of Na_v channels in the PNS requires interaction between the axon and myelinating Schwann cells [69]. Although nodal expression of $\text{Na}_v1.6$ seems to be affected under diabetic conditions [63], additional data are needed to clarify if this is related to axonal and/or Schwann cell related defects.

Post-translational modifications may also alter Na_v channel activity in DM. An increase in phosphorylation was reported previously for $\text{Na}_v1.6$, 1.7, and 1.8 in DRG neurons of diabetic rodent models – probably due to the activation of protein kinase C [62,65]. More recently, a post-translational modification of $\text{Na}_v1.8$ by methylglyoxal, a glycolytic metabolite of the AGE pathway, was reported [70]. Methylglyoxal binds to arginine residues, thereby generating a permanently active form of $\text{Na}_v1.8$ channels. It also binds to $\text{Na}_v1.7$ channels with an opposite effect, leading to their inactivation. Reactive dicarbonyls such as methylglyoxal can be detoxified by the glyoxalase system that comprises the two enzymes GLO-1 and -2. Interestingly, GLO-1 expression in the PNS is restricted to small unmyelinated peptidergic DRG neurons and axons [71]. It is therefore possible that reduced GLO-1 expression in the PNS under diabetic conditions might contribute to the DSPN phenotype in this neuronal population [71,72].

K_v channels

Much less is known about the involvement of K_v channels in DSPN, even though a decrease in K_v channel function is clearly associated with PNH [73]. K_v channels comprise 12 subfamilies (K_v1 to K_v12), of which members of K_v1 , K_v3 , and K_v7 are expressed in the PNS [11,74]. In myelinated fibers, $\text{K}_v1.1$ and $\text{K}_v1.2$ are present at the juxtaparanodes, whereas $\text{K}_v3.1b$, $\text{K}_v7.2$, and $\text{K}_v7.3$ are at the node of Ranvier. In small DRG neurons, $\text{K}_v1.4$ and $\text{K}_v7.5$ are the predominant forms in both soma and fibers (Remak bundles) (Figure 1). All are expressed in DRGs, together with minor expression of $\text{K}_v1.3$, 1.5, and 1.6.

Both K_v channel expression and function are reduced in DRG neurons of T1DM animals [75]. Recent data also demonstrated an altered distribution of juxtaparanodal $\text{K}_v1.2$ channels in T2DM *db/db* mice and T2DM patients. Interestingly, the altered $\text{K}_v1.2$ channel distribution promoted PNH in *db/db* mice [12]. Because the expression levels of $\text{K}_v1.2$ in DRG neurons and peripheral nerves were unchanged, the altered distribution is likely caused by glucose-derived post-translational modifications under diabetic conditions, a mechanism similar to that proposed for Na_v channels. Some of these modifications may be mediated by peroxynitrite. Peroxynitrite production from nitric oxide and superoxide is increased under diabetic conditions (Figure 2), and is

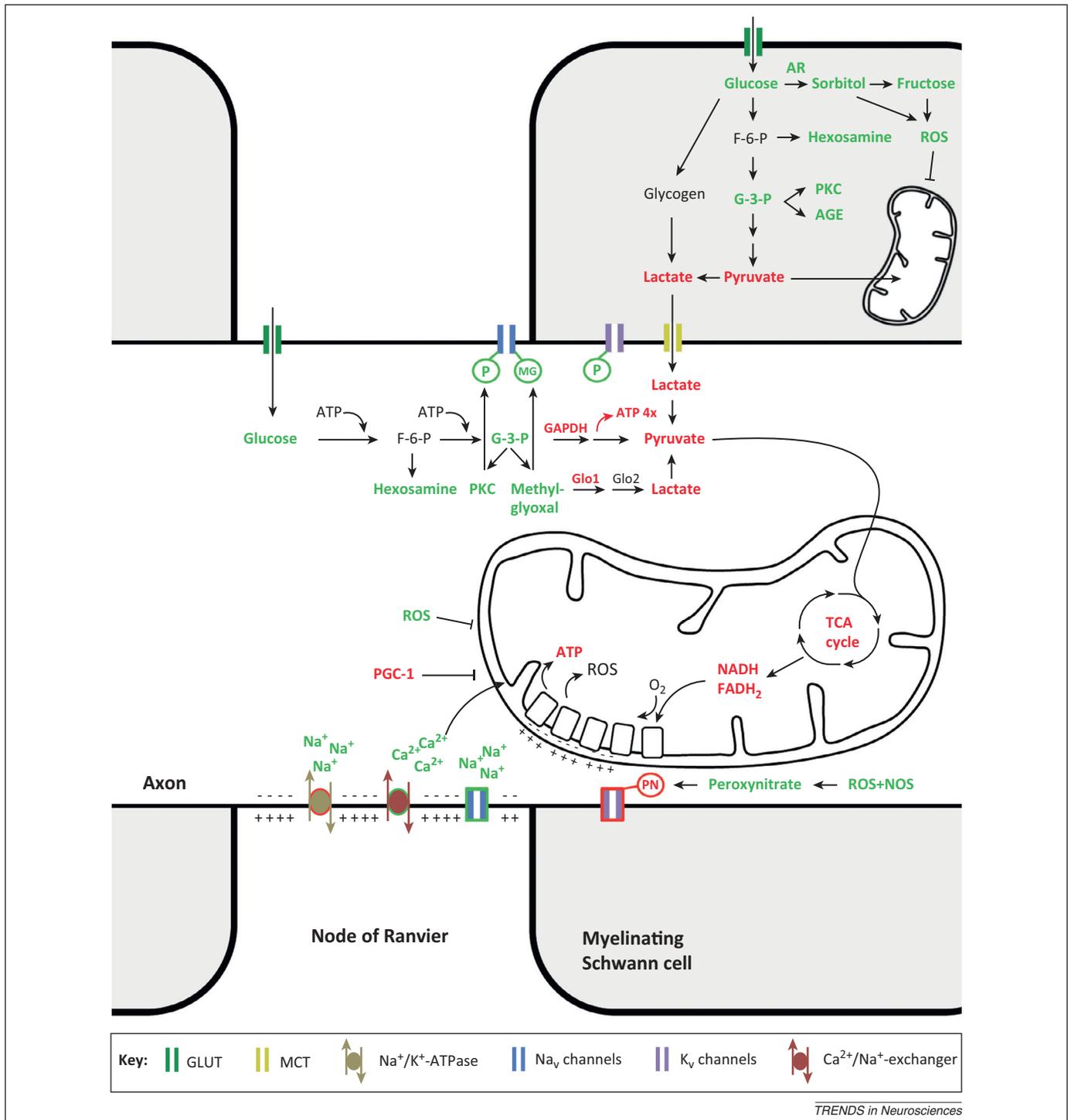


Figure 2. Consequences of glucose toxicity on mitochondrial and ion-channel function in the PNS. The nodal region of a myelinated fiber represents an area of particularly intense interactions between Schwann cells and axons. Various glial and axonal components are highlighted in green to indicate that their level/activity is increased under hyperglycemic conditions, or in red if it is decreased. Decrease in glial lactate supply (potentially mediated by MCT-dependent transport), together with decreased axonal pyruvate production under diabetic conditions, may lead to impaired capacity of axonal mitochondria to generate ATP. In addition, axonal mitochondrial function is also disturbed by increased intra-axonal generation of ROS through various pathways including hexosamine and PKC pathways, and by downregulation of the PGC-1 pathway. Consequently, reduced ATP production induces changes in nodal Na^+/K^+ -ATPase and $\text{Ca}^{2+}/\text{Na}^+$ -exchanger activity leading to variation in the axoplasmic ion composition (especially Ca^{2+} concentration), which also affects axonal mitochondrial function. In parallel, ion-channel function is also affected for both nodal Na_v (e.g., through methylglyoxal-mediated modification) and JPN K_v channels under diabetic conditions. See also the main text for a detailed description of the depicted diabetes mellitus (DM)-induced changes. Abbreviations: AGE, advanced glycation end-products; AR, aldose reductase; F-6-P, fructose 6-phosphate; G-3-P, glyceraldehyde 3-phosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Glo, glyoxalase; GLUT, glucose transporter; JPN, juxtapanode; MCT, monocarboxylate transporter; MG, methylglyoxal; P, phosphorylation; PKC, protein kinase C; PN, peroxynitrite; PNS, peripheral neural system; ROS, reactive oxygen species; PGC-1, peroxisome proliferator-activated receptor γ coactivator-1 α .

harmful to both neurons and Schwann cells [36]. Interestingly, in heart, peroxynitrite specifically nitrates tyrosine residues of $\text{K}_v1.2$ channels, and impairs $\text{K}_v1.2$ channel function, but without affecting its protein expression under

high-glucose conditions [76]. In addition, similarly to Na_v channels, K_v1 -activity is also regulated by phosphorylation and, therefore, is most likely modified by protein kinase C alterations under diabetic conditions [75] (Figure 2).

Even though Schwann cells are considered to be non-excitabile cells, they do express functional voltage-gated K_v and Na_v channels [77]. Although these channels are predominantly located in the cytoplasm, they become integrated at sites where the Schwann cell membrane is apposed to the neuronal nodal or juxtaparanodal regions that contain the neuronal Na_v and K_v channels, respectively. Further functional studies into the role of glial K_v and/or Na_v channels will be necessary to identify their contribution to the PNS function.

Possible implications for DSPN therapy

Despite great efforts, there are currently no efficient treatments for DSPN. Thus, intensive insulin diabetes therapy is regarded as the primary approach to the prevention of diabetic complications. However, studies in T1DM patients show that even intensive insulin therapy only partially prevents the development of DPN [3,78]. This may be the consequence of the continuing problem in achieving normoglycemia even with the modern forms of insulin therapy. An additional explanation for the limited efficiency of insulin diabetes therapy could be the recently described neuronal insulin resistance [21,23]. Compared to the T1DM situation, the efficiency of intensive diabetes therapy in preventing or retarding DSPN in T2DM patients is controversial [3].

Based on the various pathogenetic mechanisms mentioned above, several agents have recently been evaluated in randomized clinical trials. These include AR inhibitors (e.g., alrestatin, sorbinil, ponalrestat, tolrestat, epalrestat, zopolrestat, zenarestat, fidarestat, ranirestat), the antioxidant α -lipoic acid (also known as thioctic acid), essential fatty acids (e.g., γ -linolenic acid), AGE inhibitors (e.g., tran-dolapril), prostacyclin analogs (e.g., iloprost, beraprost), prostaglandin derivatives (e.g., PGE1- α -cyclodextrin), NGF, a PKC β inhibitor (ruboxistaurin), C-peptide, vascular endothelial growth factor (VEGF), benfotiamine, and actovegin (an ultrafiltrate derived from calf blood, comprising more than 200 physiological components). However, so far, only the antioxidant α -lipoic acid, benfotiamine (a vitamin B1 derivative), actovegin, and epalrestat are licensed and used for treatment of DSPN [5,79,80]. Importantly, a controlled, large, long-term clinical trial, which is critical for the proper evaluation of the progression of DSPN [81], has only been carried out for α -lipoic acid, and showed a favorable effect on neuropathic impairment in diabetic patients with mild to moderate DSPN [82].

All drugs listed above have been designed to influence favorably the underlying neuropathic process contributing to DSPN. In addition, multiple therapeutic approaches are available to manage pain in diabetic patients [2,3], but none

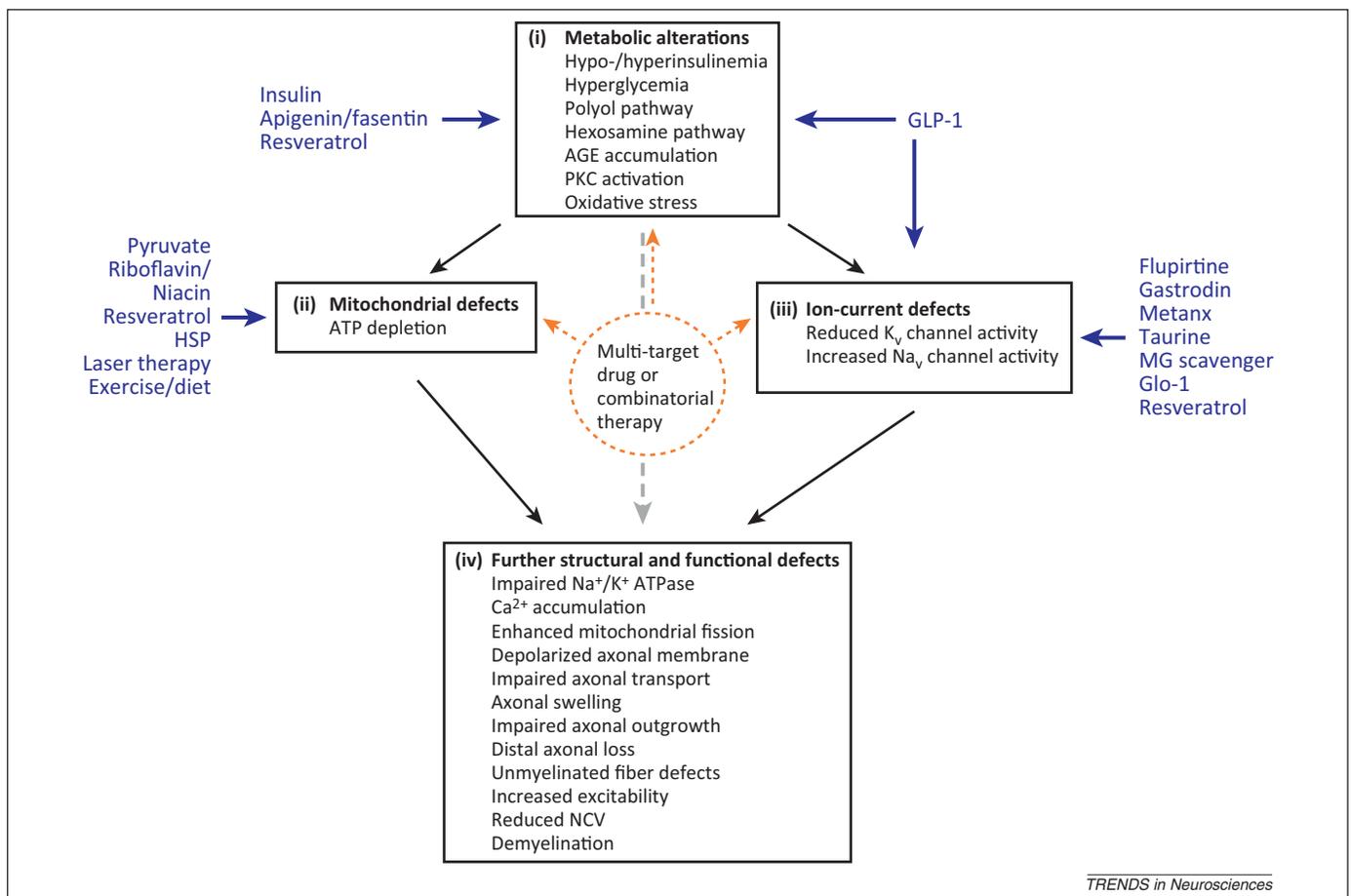


Figure 3. Potential approaches to prevent diabetic sensorimotor polyneuropathy (DSPN) consequences in neurons and glia. Various alterations induced by diabetes mellitus (DM), which all potentially affect peripheral neural system (PNS) function, are listed. The chronology of possible pathological events contributing to DSPN includes initial metabolic alterations (i), which affect mitochondrial ATP production (ii) and ion-channel function (iii), as well as possible alternative pathways (represented by gray dotted line) leading to further structural and functional defects of the axon–glial network (iv). Drugs shown to regulate glucose metabolism and to reverse mitochondrial or ion-channel function are represented in blue and the desirable effects of future multi-target drugs and/or combinatorial therapy are indicated in orange. Abbreviations: Glo, glyoxalase; HSP, heat shock protein; MG, methylglyoxal; NVC, nerve conduction velocity; PKC, protein kinase C.

prevents the development of pain under DM. Thus, from the clinical point of view, there is a continuing need for the development of novel drugs tailored to target pathogenetic mechanisms underlying DSPN. The advantage of such treatment approaches is that they may exert their effects despite prevailing hyperglycemia. Mitochondrial dysfunction (particularly associated with ATP depletion) may play an important role in the detection and potential treatment of DSPN (Figure 3). Multiple *in vitro* approaches permit the evaluation, directly or indirectly, of mitochondrial function (e.g., ATP synthesis and cellular redox homeostasis) [83]. Recent progress in the use of magnetic resonance spectroscopy as a non-invasive method to measure ATP production may provide an interesting alternative for evaluating mitochondrial function *in vivo* [83]. Importantly, enhancement of mitochondrial function could potentially be achieved with resveratrol and/or overexpression of heat-shock proteins, two treatments previously shown to improve DSPN in diabetic animal models [84–86]. Because the diffusion of ATP is low, other energy-rich molecules, such as sodium pyruvate (which was shown to stimulate mitochondrial function [87]), may provide an interesting alternative in compensating for ATP depletion. In addition, commercially available vitamin B derivatives, riboflavin and niacin, precursors of NADH and FADH₂, as well as coenzyme Q10, have been shown to improve mitochondrial function in cancer [88]. Further studies will be needed to evaluate the efficacy of these ‘energy-modulating’ vitamins in DSPN. Moreover, light as a source of energy applied through non-invasive low-level laser therapy on various tissues, including normal human neuronal progenitors [89] or human Schwann cells [90], resulted in increased ATP production or cell proliferation, respectively. Additional *in vivo* testing will be needed to determine the tolerable limit of laser power and its effectiveness. It was also recently demonstrated that neuronal mitochondrial function defects in T1DM mice could be partially improved by treatments with ciliary neurotrophic factor [91]. DSPN affects both myelinated and unmyelinated fibers. The latter are estimated to consume 2.5- to 10-fold more energy per generated AP [92], suggesting that they may in particular benefit from potentially improved mitochondrial function. However, because the energy consumption of an axon also depends on its firing frequency, which is usually higher in thicker axons [93], all axonal populations might represent good targets for therapies aiming at improved axonal energy supply.

In addition to ATP deficiency, ion channels are impaired under diabetic conditions and may prove to be good drug targets of specific importance for the PNS (Figure 3). As a consequence of post-translational modifications, their activity is either inhibited (K_v channels) or activated (Na_v channels). Scavengers for the causative reactive molecules (in particular methylglyoxal and peroxynitrite) partially improve DSPN phenotypes as shown for metanx [94], taurin [95], MG-Scavenger, or Glo-1 overexpression [70,71]. It was also previously shown that the K_v7 channel activator flupirtine can compensate for the reduced K_v1 current and decreases PNH [12]. Similar results have been obtained with gastrodin, which normalizes both the K_v and Na_v currents [57]. The use of subtype-specific modulators for Na_v and K_v channels that are predominantly present in the PNS might

Box 3. Outstanding questions

- Do Schwann cells develop insulin resistance similarly to neurons? If so, which Schwann cell functions are affected?
- Is GLUT localization, and therefore function, in neurons and/or in SCs affected under diabetic conditions?
- Which MCTs are involved in axon–glial interaction in the PNS? Are they also clustered at nodal areas? Is their localization/function affected by diabetic conditions?
- Where are IR, GLUTs, and MCTs localized in axons enwrapped by nonmyelinating Schwann cells?
- To what extent is the CNS protected from the DM-induced changes potentially affecting axon–glial interactions in the PNS?
- Are mechanisms involved in transport/anchoring of ion channels affected by DM? Could these mechanisms also provide DPN drug targets with limited side effects?
- What is the role of Na_v and K_v channels present in Schwann cells? Are they also affected by diabetic conditions?
- Other neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), and Friedreich ataxia, are also believed to be caused by mitochondrial dysfunction. Is there a common mechanism underlying these disorders and DM?

be of particular interest to limit side effects. Recently, 4,9-anhydro-tetrodotoxin has been described to inactivate Na_v1.6 specifically but has not been tested in DSPN conditions [96]. Interestingly, the above-mentioned resveratrol, which improves DSPN phenotypes by capturing peroxynitrite, can also inhibit Na_v channels [97], improve mitochondrial function via the activation of Sirt1 and PGC-1 α [98], and attenuate insulin resistance and the activation of Glo-1 [99].

The aim of preventing the appearance, or slowing the progression, of DSPN phenotypes will probably require a combinatorial strategy addressing different pathophysiological mechanisms involved in DSPN (Figure 3). In addition to modulating mitochondria and ion channels, regulation of glucose transporters may also provide a means to limit the consequences of hyperglycemia in the PNS. Two inhibitors of GLUT-1-mediated glucose uptake, apigenin [100] and fasentin [101], were successfully used on cancer cells. However, the efficiency of this approach in the PNS needs to be verified.

Concluding remarks

Although neurons and their associated axons are clearly the central component of the PNS, there is a growing body of evidence indicating that their function and maintenance under normal and disease conditions depend critically on adjacent Schwann cells. Both Schwann cell function and interaction with the underlying axons play a role in axonal energy metabolism and ion conductance, two processes that are affected in DSPN. The evidence available so far indicates that nodal areas of peripheral nerves may have a particular role in DSPN pathology. Nodes are regions of intense interactions between Schwann cells and axons, and the majority of players implicated in DSPN (e.g., IR, GLUTs, Na_v and K_v channels, and mitochondria) predominantly localize there. Even though the mechanisms underlying defects in the axon–glia network remain to be clarified (Box 3), the current data may provide new ideas for hypothesis-driven therapeutic approaches aimed at preventing or delaying DSPN.

Acknowledgments

We would like to apologize to our colleagues whose work is not cited owing to length restrictions. We thank the Swiss National Science Foundation for funding support. We would particularly like to thank Dr Hasna Baloui and Patrick Burrola for providing the electron microscopy pictures used in Figure 1, and Drs Olivier Poirot, Enric Domènech-Estévez, and Mark Verheijen for critical reading of this manuscript. Finally, we would like to thank current and past members of the Chrast laboratory for stimulating discussions.

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