Published in final edited form as:

Int Rev Neurobiol. 2016 ; 127: 211–225. doi:10.1016/bs.irm.2016.03.005.

Treating painful diabetic neuropathy: Preventing pain vs. blocking pain by targeting nociceptive ion channels in sensory neurons

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Abstract

Pain-sensing sensory neurons (nociceptors) of the dorsal root ganglia (DRG) and dorsal horn (DH) can become sensitized (hyperexcitable) in response to pathological conditions such as diabetes, which in turn may lead to the development of painful peripheral diabetic neuropathy (PDN). Because of incomplete knowledge about the mechanisms underlying painful PDN, current treatment for painful PDN has been limited to somewhat non-specific systemic drugs that have significant side effects or potential for abuse. Recent studies have established that several ion channels in DRG and DH neurons are dysregulated and make a previously unrecognized contribution to sensitization of pain responses by enhancing excitability of nociceptors in animal models of Type 1 and Type 2 PDN. Furthermore, it has been reported that targeting post-translational modification of nociceptive ion channels such as glycosylation and methylglyoxal metabolism can completely reverse mechanical and thermal hyperalgesia in diabetic animals with PDN *in vivo*. Understanding details of post-translational regulation of nociceptive channel activity may facilitate development of novel therapies for treatment of painful PDN. We argue that pharmacological targeting of the specific pathogenic mechanism rather than of the channel per se may cause fewer side effects and reduce the potential for drug abuse in patients with diabetes.

Keywords

T-type channels; calcium; GABA; TRPV1; hyperalgesia; allodynia; diabetes; nociceptors

Introduction

Diabetes mellitus is a chronic, multifactorial disease characterized by hyperglycemia. The World Health Organization estimates that 220 million people worldwide currently suffer from diabetes. Diabetes mellitus type 1 (formerly called insulin-dependent diabetes or juvenile diabetes) is a form of diabetes that results from autoimmune destruction of insulin-producing β -cells in the pancreas. In contrast, type 2 diabetes (formerly called noninsulin-dependent or adult-onset diabetes), the most common form of diabetes, results from insulin resistance and is most often associated with obesity. Peripheral diabetic neuropathy (PDN) is

the most frequent complication of diabetes and The Center for Disease Control and Prevention estimates that 60%–70% of diabetic patients will develop PDN symptoms with prevalence increasing with the duration of diabetes mellitus (Centers for Disease Control and Prevention, 2011). The prevalence of painful PDN is often underestimated, but a recent large community-based study with more than 15,000 patients with diabetes reported painful symptoms in about one third of all participants (Abbott et al., 2011). These patients can suffer from both stimulus-evoked painful episodes, including hyperalgesia (exaggerated pain experience upon presentation of noxious stimuli) and/or allodynia (normally non-noxious stimuli that may evoke pain sensation), as well as spontaneous pain in the form of burning or tingling. Interestingly, these painful pathologies in diabetics usually occur in parallel with degeneration of peripheral nerves (Abbott et al., 2011). Eventually, these painful symptoms usually subside as the disabling pain is replaced by complete loss of sensation (insensate PDN). Both intractable pain and loss of sensation have significant adverse effects on quality of life measures. Unfortunately, current treatment options are unable to reverse these symptoms and are often associated with serious side effects. For example, $\text{Ca}_v2.2$ (N-type) subtypes of high-voltage-activated calcium channels and their regulatory subunit $\alpha_{2\delta}$ are considered a major cellular target for the anticonvulsants gabapentin and pregabalin, which are commonly used to relieve diabetes-induced pain in clinics. However, more than 50% of patients using gabapentin or pregabalin experience side effects, such as excessive sedation, ataxia, dizziness, euphoria, and weight gain, all of which limit their clinical use (Edwards et al., 2008). Although opioids are partially effective for treatment of painful PDN, they are not suitable for chronic use since their long-term use is associated with serious side effects including constipation, urinary retention, impaired cognitive function, respiratory depression and issues associated with tolerance and addiction. Furthermore, topical applications of therapeutic creams with lidocaine and capsaicin are inconsistently effective in patients with painful PDN and cause unpleasant side effects like numbness and burning respectively. This underscores the fact that new mechanism-specific therapeutic treatments must be considered that could be more effective and possibly better tolerated in diabetic patients.

Pain is often a result of the activation of neurons (nociceptors) that are activated following injury of peripheral tissue such as the skin and internal organs. This form of pain, called nociceptive pain, is self-protective and typically evokes actions aimed at limiting or avoiding further tissue damage. Nociceptive pain is typically responsive to treatments with conventional painkillers, such as non-steroidal anti-inflammatory drugs (NSAIDs) or opiates. In contrast, neuropathic pain results from primary dysfunction of peripheral nociceptive, as well as non-nociceptive nerves and/or components of the central nervous system (“central pain”). Neuropathic pain typically outlasts the initial stage of injury and frequently leads to debilitating disorders that are not amenable to conventionally available drug therapies. It is generally accepted that pathologies associated with neuropathic pain result from increased excitability of peripheral nerves and/or pain pathways in central nervous system (CNS). For example, nociceptors can become inappropriately sensitized by various mechanisms in response to local or systemic metabolic conditions or peripheral tissue injury associated with diabetes. Multiple pathogenic mechanisms, such as the formation of intracellular advanced glycation end products (AGE), release of inflammatory cytokines, increased glucose metabolism by the polyol pathway and oxidative stress may

contribute to the impaired function of sensory neurons in animals with PDN. However, many preclinical and clinical studies aiming to target several of these mechanisms while simultaneously ensuring proper blood glucose control were unable to provide definite and complete pain relief (Edwards et al., 2008; Gooch and Podwall, 2004). This incomplete pain reversal strongly suggests that other molecular mechanisms may be involved in hyperglycemia-induced alterations of function of nociceptive sensory neurons.

Nociceptive ion channels control neuronal excitability

Several studies conducted in recent years have reported on the plasticity of various ion channels expressed in nociceptive sensory neurons, also known as dorsal root ganglion (DRG) neurons, which play a critical role in modulating overall cellular excitability (reviewed by Calcutt, 2013; Todorovic and Jevtovic-Todorovic, 2014). These findings are both important and relevant, since increased excitability of sensory neurons is believed to contribute directly to the development and maintenance of painful symptoms, including hyperalgesia, allodynia, and spontaneous pain. Studies have shown that the Cav3.2 isoform of the T-type voltage-gated calcium channel (T-channel) is heavily expressed in DRG cells and dorsal horn (DH) of the spinal cord and plays a distinct role in supporting pathological pain in animal models of both type 1 and type 2-induced PDN (Latham et al., 2009; Jacus et al., 2012; Jagodic et al., 2007). T-type channels were first discovered in preparations of acutely dissociated sensory neurons of small size (Carbone and Lux, 1984). The characteristic biophysical features, including activation by small membrane depolarization, relatively fast and voltage-dependent inactivation kinetics, as well as slow deactivation kinetics upon channel closing following maximal activation, make them ideally suited to control cellular excitability of sensory neurons. Relatively soon after their initial discovery, it was indeed demonstrated that T-channels control sub-threshold excitability of DRG cells by lowering the threshold for action potential generation in a subpopulation of acutely dissociated rat DRG cells of medium size (White et al., 1989). By convention, it is accepted that most of the smaller diameter DRG cells (cell soma < 31 μm) are putative nociceptors, while medium size DRG cells (cell soma 32–45 μm) can be either nociceptive or non-nociceptive sensory neurons. Most acutely dissociated small DRG cells expressing T-currents are sensitive to the algogenic compound from hot peppers, capsaicin, and have the electrical properties of classically described nociceptors with wide action potentials (Nelson et al., 2007). T-channels are also present in a subpopulation of medium sized acutely dissociated sensory neurons that are either putative nociceptors, since they bear the IB₄ (isolectin B₄) antigen (Jagodic et al., 2007), or that subserve touch sensation mediated by specific DRG cell type called D-hair mechanoreceptors (Dubreuil et al., 2004; Shin et al., 2003). Furthermore, the Ca_v3.2 isoform of T-channels is a critical regulator of cellular excitability of nociceptive DRG neurons termed “T-rich” cells that are also responsive to capsaicin and are IB₄ positive (Nelson et al., 2005). Prominent Ca_v3.2 T-currents in “T-rich” and medium size DRG cells generate visible after-depolarizing potentials (ADP) and high-frequency burst firing along with small depolarizations of the membrane potential. In addition, amplification of sub-threshold membrane depolarizations *via* Ca_v3.2 T-channels typically decreases the threshold for action potential firing in DRG cells that express T-channels.

Alterations of voltage-gated and ligand-gated ion channels in sensory neurons in animal models of type 1 diabetes with painful PDN

Several studies have demonstrated up-regulation of $\text{Ca}_v3.2$ currents in both small and medium size DRG cells in animal models of painful PDN in type 1 diabetes. For example, in diabetic Bio-Bred/Worcester (BB/W) rats, a model for autoimmune-mediated type 1 diabetes, up-regulation of T-channel current density of about three-fold was observed in a mixed population of small and medium size DRG cells after 8 months in a diabetic state (Hall et al., 1995). The current-voltage relationship in these cells was not different between control, healthy rats and experimental diabetic rats. Similarly, studies with streptozotocin (STZ)-induced diabetic neuropathy, which used electrophysiological recordings from acutely isolated IB₄-positive medium and small size DRG cells, found that T-current amplitude was up-regulated about two-fold (Cao et al., 2011; Jagodic et al., 2007; Khomula et al., 2013). In addition, these three studies have shown that biophysical properties of T-channels were altered in DRG cells from diabetic animals, with a significant depolarizing shift in steady-state inactivation curves. As a result of this shift, more T-channels were available at physiological membrane potentials, and this increased propensity for burst firing in DRG cells from diabetic rats as compared with healthy ones (Jagodic et al., 2007; Khomula et al., 2013).

Additional studies have documented up-regulation of pro-nociceptive ion channels such as voltage-gated sodium channels, particularly the $\text{Na}_v1.7$ and $\text{Na}_v1.8$ isoforms (Hoeijmakers et al., 2014; Bierhaus et al., 2012), purinergic P2X₃ receptors (Xu et al., 2011), and the vanilloid family of ligand-gated channels, especially transient receptor potential vanilloid 1, TRPV1 (Brederson et al., 2013), within sensory neurons in animal models of diabetes. Given the prominent role of TRP channels in sensory transduction, it is reasonable to assume that dysregulation of capsaicin-gated TRPV1 channels may also contribute to abnormal pain signaling in different subpopulations of DRG cells from rats with STZ-induced PDN. For example, Hong and Wiley found using immunohistochemistry that membrane expression of TRPV1 protein in rats was increased in myelinated A-type fiber DRG cells, while it was decreased in small unmyelinated C-type nociceptive DRG cells as a result of diabetes (Hong and Wiley, 2005). In contrast, a study by Pabbidi and colleagues demonstrated increased expression of TRPV1 protein and greater whole-cell current in small DRG cells in both STZ-induced and transgene-mediated type 1 painful PDN in mice (Pabbidi et al., 2008). Consistent with increased function of TRPV1 in DRG cells expressing T-currents, capsaicin-gated currents were increased in medium-size (Jagodic et al., 2007) and small DRG cells (Khomula et al., 2013; Shankarappa et al., 2011) from STZ-induced diabetic rats with painful PDN. This observation underscores the importance of possible cross-talk between nociceptive ligand and voltage-gated ion channels that control calcium signaling in DRG cells. Increased activity of both channel types in concert could contribute to cellular hyperexcitability and consequently to hyperalgesia and allodynia in PDN. Pabbidi and colleagues reported that STZ may have had a direct effect on expression of TRPV1 channels in DRG cells that was independent of hyperglycemia and hence may result from the direct toxicity of this compound (Pabbidi et al., 2008). However, present evidence demonstrates that this is not the case with DRG $\text{Ca}_v3.2$ T-channels. A causal relationship between hyperglycemia in STZ-induced PDN in rats and expression of T-type currents in

DRG cell was effectively documented since up-regulation of T-currents closely followed development of hyperglycemia and was completely reversed in parallel with diabetic hyperalgesia reversal upon chronic insulin treatments (Messinger et al., 2009). An interesting report has documented that forced exercise in diabetic STZ-treated rats prevented the depolarizing shift in steady-state inactivation of T-currents in small DRG cells in parallel with improvement of pain symptoms of PDN *in vivo* (Shankarappa et al., 2011). This is important given that these findings could potentially be translated in human medicine. Furthermore, *in vivo* knock-down of the Ca_v3.2 isoform of T-channels in DRG cells using antisense oligonucleotides reversed up-regulation of T-currents in small DRG cells and diabetic hyperalgesia in STZ-treated rats (Messinger et al., 2009; Obradovic et al., 2014). Finally, as proof-of-concept, it was reported that when compared to wild-type littermates, diabetic STZ-treated knockout mice lacking the Ca_v3.2 isoform of T-channels failed to develop thermal and mechanical hyperalgesia despite the prominent hyperglycemia (Latham et al., 2009). Taken together, these studies have firmly established that the Ca_v3.2 isoform of T-channels in DRG cells is required for the development and maintenance of painful PDN in rats and mouse models of type 1 diabetes.

Alterations of Ca_v3.2 T-type channels in nociceptive sensory neurons in animal models of type 2 diabetes with painful PDN

Morbid obesity may be accompanied by type 2 diabetes and painful PDN that is manifested by mechanical/thermal allodynia and hyperalgesia. Importantly, clinical studies have documented that painful PDN is at least two-fold more common in patients with type 2 PDN than in patients with type 1 PDN (Edwards et al., 2008). Hence, the function of Ca_v3.2 T-channels in DRG cells and its possible relationship with abnormal pain perception in PDN was investigated in leptin-deficient morbidly obese ob/ob mice (Latham et al., 2009). It was found that ob/ob mice developed significant mechanical and thermal pain hypersensitivity early in life that coincided with the onset of hyperglycemia (maximal at the age of 10–16 weeks) and was reversed with timely administered insulin therapy that was initiated before diabetes fully developed. These disturbances were accompanied by significant biophysical and biochemical modulation of T-channels in DRG neurons as indicated by about a two-fold increase in the amplitude of T-currents and about a three-fold increase in expression of channel mRNA. The most prevalent isoform of T-channels expressed in nociceptive DRG cells, Ca_v3.2, was the most strongly affected. Moreover, systemic injections of (3β,5α, 17β)-17-hydroxyestrane-3-carbonitrile (ECN), a neuroactive steroid compound and selective T-channel antagonist, provided dose-dependent alleviation of neuropathic thermal and mechanical hypersensitivity in diabetic ob/ob mice. This study indicates that selective pharmacological antagonism of T-channels can potentially be an important novel therapeutic approach for the management of pain associated with PDN. Thus, it appears that in both type 1 and type 2 painful PDN, metabolic abnormalities leading to hyperglycemia may be causative events that eventually lead to up-regulation of DRG T-current, increased cellular excitability of nociceptors and consequently hyperalgesia and allodynia.

Post-translational modification of pro-nociceptive ion channels in PDN

Aberrant regulation of function of nociceptive ion channels in sensory neurons *via* post-translational modification and ensuing hyperexcitability may be factors contributing to painful symptoms of PDN. One of the most prevalent forms of post-translational modification of proteins is glycosylation, which involves the attachment of sugar molecules to a protein. Glycosylation regulates protein conformation and activity, is involved in protein protection from proteolytic degradation, and plays an important role in protein trafficking and secretion (Moremen et al., 2012). However, very little is known about the possible involvement of glycosylation in the sensitization of nociceptive responses in the setting of diabetic hyperglycemia. Unsurprisingly, it has been reported that diabetes results in a nearly three-fold increase in peripheral nerve glycosylation as measured in tissue homogenates from diabetic rats and dogs (Vlassara et al., 1981). Hence, it is plausible that aberrant and/or excessive glycosylation of certain susceptible proteins in peripheral nerves may play a role in the pathogenesis of PDN. Unfortunately, the identity of these glycosylated proteins and their precise role in the pathogenesis of diabetes-induced nerve dysfunction remain elusive.

Protein glycosylation frequently involves extracellular asparagine residues. Thus, it is plausible to propose that as glucose levels rise in sensory neurons, as a consequence of diabetic hyperglycemia, excessive glycosylation may become a maladaptive process and lead to dysfunction of certain ion channels that control cellular excitability. Prevailing literature has focused on protein glycation in a diabetic environment, a process that typically involves intracellular accumulation of advanced glycation end products (AGE) that alter protein function by modifying intracellular arginine and lysine residues (Jack and Wright, 2012; Obrosova (2009). However, decreasing production of AGE *in vivo* has inconsistently and incompletely reversed pain symptoms in animal models of diabetes and in patients with PDN (Edwards et al., 2008). Hence, it is possible that other parallel signaling mechanisms, such as glycosylation, may be involved in hyperglycemia-induced alterations of nociceptive ion channel function in sensory neurons.

Up-regulation of Ca_v3.2 (but not Ca_v3.1 or Ca_v3.3) transcripts in DRG tissues from ob/ob mice can account at least partially for increased T-current density in small DRG cells, but it does not account for kinetic alterations of T-currents (Latham et al., 2009). Thus, it was considered that these channels in DRGs from diabetic ob/ob mice must be additionally modified by other mechanisms including glycosylation. This was recently addressed by using patch-clamp recordings from small DRG and recombinant human embryonic kidney (HEK-293) cells, immunohistochemistry, biochemical studies, as well as *in vivo* pain studies using ob/ob mice (Orestes et al., 2013). First, it was found that, in addition to increased current density, T-currents in small DRG cells exhibit marked speeding of macroscopic current kinetics in diabetic ob/ob mice. The glycosylation inhibitor neuraminidase completely reversed up-regulation and kinetic alterations of Ca_v3.2 in small DRG cells from diabetic ob/ob mice while it had a much smaller effect on T-currents in healthy wild-type mice. Intra-plantar (i.pl.) injections of neuraminidase directly into peripheral receptive fields of diabetic ob/ob mice were able to reverse completely the mechanical and thermal hyperalgesia. To test whether the anti-hyperalgesic effect of neuraminidase in ob/ob mice is related to its effects on T-currents, the selective T-channel blocker ECN was injected and

completely reversed thermal and mechanical hyperalgesia in ob/ob mice while subsequent i.pl. injections of neuraminidase in paws did not further decrease pain thresholds. Hence, these studies indicate that glycosylation inhibitors like neuraminidase may be used to normalize sensory transmission in DRG cells and peripheral nerves in animals with painful PDN.

Two recent independent studies have reported that glycosylation of critical extracellular asparagine residues of Ca_v3.2 channels causes kinetic alterations, up-regulation of T-currents, as well as an increase in membrane expression of T-channels (Orestes et al., 2013; Weiss et al., 2013). Molecular studies using site-directed mutagenesis identified conserved extracellular asparagine residues, most notably N192 and N1466, as important regulators of Ca_v3.2 current kinetics and channel membrane expression, respectively. The involvement of N192 was supported by patch-clamp recordings that demonstrated slower current kinetics in an N192Q Ca_v3.2 mutant, with apparently normal membrane expression. Interestingly, T-currents in HEK-293 cells transfected with an N1466Q Ca_v3.2 mutant could not be consistently recorded and imaging studies showed minimal membrane expression of this mutant (Orestes et al., 2013). Thus, different glycosylation sites in Ca_v3.2 channels may have distinct functional roles. Surprisingly, authors of both studies could not record T-currents from N271Q Cav3.2 channels despite their presence in the cell membrane. It remains possible that N271Q channels trafficked to the membrane are nonfunctional. In contrast to the finding of Orestes et al., Weiss and colleagues found that asparagine N192 serves as a regulator of Ca_v3.2 channel membrane expression and asparagine N1466 as a regulator of channel kinetics (Weiss et al., 2013). It is possible that different levels of glucose in cell culture could have contributed to the different findings between the studies. Glycosylated products have larger molecular mass, meaning that glycosylation or the enzymatic removal of these groups from the channel protein will induce a shift in the migration of proteins bands resolved by gel-shift analysis and visualized by Western blotting. The study by Orestes and colleagues took advantage of this and directly demonstrated, using gel-shift analysis, that recombinant Ca_v3.2 channels are, indeed, glycosylated within domain I of the channel protein. However, levels of glycosylation of native Ca_v3.2 channels in sensory neurons in diabetic animals remain to be determined in future studies. Finally, neither of these two studies examined the role of neuraminidase or other agents that can modulate glycosylation upon membrane excitability of nociceptors in animals with PDN.

Several other *in vitro* studies have reported that glycosylation may modulate properties of other pro-nociceptive voltage-gated ion channels. For example, it was reported that in embryonic DRG neurons, neuraminidase alters steady-state inactivation of voltage-gated sodium channels (Tyrell et al., 2001). This study did not find any effects of neuraminidase on voltage-gated sodium channels in small DRG cells from healthy adult animals. However, it remains to be determined in future studies if neuraminidase could modulate voltage-gated sodium channels in nociceptive DRG cells from diabetic animals. Future extensive electrophysiological studies could also be expanded to involve examination of other ligand-gated channels (e.g. TRPV1) that are crucial for the control of cellular excitability of DRG cells from diabetic animals that might also be modulated by glycosylation (Jing et al., 2012; Pertusa et al., 2012; Cohen, 2006).

In addition to glycosylation of nociceptive ion channels, post-translational modification of the voltage-gated sodium channels, specifically Nav1.8 isoform and transient receptor potential channel A1 (TRPA1), *via* methylglyoxal, an endogenous reactive metabolite of glucose, has been demonstrated in DRG neurons in animals with STZ-induced diabetes (Bierhaus et al., 2012; Eberhardt et al., 2012; Anderson et al., 2013). High plasma concentrations of methylglyoxal correlate well with occurrence of painful symptoms in patients with PDN (Bierhaus et al., 2012). Furthermore, methylglyoxal depolarizes putative nociceptive DRG neurons and induces hyper-excitability of these cells thus facilitating action potential firing and *in vivo* administration of methylglyoxal evoked thermal and mechanical hyperalgesia. In contrast to glycosylation, above mentioned studies have demonstrated that this form of post-translational modification of Nav1.8 channels and TRPA1 channels is *via* modification of intracellular binding sites (e.g. lysine, arginine) (Bierhaus et al., 2012; Eberhardt et al., 2012).

Finally, up-regulation of high-voltage-activated (HVA) calcium currents in sensory neurons in STZ-treated diabetic rats *via* G-proteins may underlie their diminished responsiveness to opioids (Hall et al., 1996; 2001).

Diminished inhibitory drive in the spinal cord may also contribute to painful PDN

Activation of nociceptors can trigger a lasting increase in the excitability and synaptic efficacy of dorsal horn (DH) neurons in a phenomenon termed central sensitization, which in turn contributes to many pain disorders in humans including: neuropathic pain, fibromyalgia and osteoarthritis (Woolf, 2011). This can be manifested as prolonged and/or exaggerated response to painful stimuli (hyperalgesia), a reduction in threshold for pain (allodynia), and/or receptive field expansion that enables input from non-injured tissues to produce pain (secondary hyperalgesia). Hence, drugs like gabapentin and pregabalin that reduce central facilitation of DH neurons by diminishing glutamate-mediated excitatory drive, *via* binding to the $\alpha 2\delta$ ancillary subunit of voltage-gated calcium channels, are among the most effective treatments for hyperalgesia and allodynia in PDN. Unfortunately, in many patients (about 50%) these drugs induce sedation, making them unsuitable for long-term therapy (Gooch and Podwall, 2004). Furthermore, it is well documented that promotion of inhibitory drive mediated *via* γ -aminobutyric acid (GABA) activation in the DH to activate inhibitory inputs is also beneficial in alleviating neuropathic pain symptoms in various animal models of peripheral nerve injury (Syvilotti and Wolf, 2004; Woolf 2011). Surprisingly, the possibility that spinal GABAergic system may be involved in painful PDN has not been extensively studied. In a very interesting recent study, Lee-Kubli and Calcutt (2014) have found diabetic rats have impaired spinal GABA_A receptor inhibitory function arising from reduced spinal potassium chloride co-transporter, which collectively leads to spinal disinhibition of pain responses. Hence, designing therapies that aim to restore impaired GABAergic function in spinal cord may be a novel approach to the treatment of painful PDN.

Conclusions

It is likely that multiple signaling pathways targeting nociceptive ion channels may work in concert to promote hyperexcitability of sensory neurons and contribute to hyperalgesia, allodynia, and spontaneous pain in patients with PDN. We summarized here how different pro-nociceptive ion channels expressed in peripheral nerve terminals in skin, cell somas in DRG, and central terminals of sensory neurons, may work together with spinal GABA-mediated dysfunction to increase sensitization of pain responses under diabetic conditions. It is hoped that future efforts in targeting metabolic pathways such as glycosylation and/or methylglyoxal formation may lead to novel and safer pain therapies in patients with diabetes. We posit that a therapeutic strategy to suppress pain by normalizing nociceptive channel function will be advantageous to that of direct channel blockade because, by correcting the pathology of PDN at its source, this approach should avoid the multitude of unintended side effects that are associated with current therapies. We envision that specific targeting of dysregulated biochemical pathways that regulate function of nociceptive ion channels may be developed into a novel, effective and safe mechanistic-based therapeutic approach. This may be achieved by specifically targeting peripheral axons of nociceptors using therapeutic ointments and creams and/or applying drugs that reverse cellular hyperexcitability directly onto DRGs using fluoroscopy-guided epidural injections in pain clinics. Alternatively, systemically administered drugs may be used, although this will likely increase the risk of side effects. Most patients with painful PDN ask for medical help well after painful symptoms have developed. It is an attractive alternative approach to attempt to identify the population at risk for painful PDN using screening test for glycosylated and methylglyoxal-modified proteins in patients with PDN. This would allow physicians to initiate treatments before sensitization of peripheral and central pain responses is fully developed. To our knowledge, this topic is controversial and a recent clinical study failed to find correlation between serum methylglyoxal levels and symptoms of peripheral and cardiovascular autonomic neuropathy (Hansen et al., 2015). However, studies like this remain an important area of future investigations.

Acknowledgments

Our research is supported by American Diabetes Association National Award for Basic Research 7-09-BS-190 (to S.M.T.) and research funds from the Department of Anesthesiology at the University of Virginia, Charlottesville, VA, as well as Department of Anesthesiology at the University of Colorado Anschutz Medical Campus, Aurora, CO.

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