

## Chapter 2

# Biology and Pathophysiology of Painful Diabetic Neuropathy

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### Clues from Diabetic Patients

The complexity of the diabetic condition, compounded by difficulties in defining and quantifying neuropathy and pain, means that the pathogenic cascades linking diabetes to neuropathy and neuropathy to pain remain poorly understood. Nevertheless, clinical studies that have highlighted associations in patients with diabetes and neuropathic pain may offer some insight into potential causal mechanisms.

### *Diabetes and Neuropathy*

Diabetes mellitus represents a syndrome of complex metabolic diseases united by the occurrence of hyperglycemia which also exhibits diverse origins (insulin deficiency, insulin resistance, systemic consequences of pregnancy) and has variable accompanying physiological and metabolic disorders. As neuropathy and neuropathic pain occur in both type 1 and type 2 diabetic patients independent of circulating insulin levels, the majority of clinical and experimental investigations have started from the premise that hyperglycemia is the primary cause of neuropathy and neuropathic pain. In support of this approach, studies that followed large cohorts of diabetic patients over many years established that neuropathy is the most frequently occurring complication of diabetes and that duration of diabetes and poor long-term glycemic control are major risk factors for neuropathy [1, 2]. However, it is also important to recognize that around half of all diabetic patients in these studies

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never develop detectable neuropathy, while the correlation between hyperglycemia and neuropathy is not overwhelming. Thus, while improving glycemic control has to be the cornerstone of prophylactic therapy to prevent neuropathy in diabetic patients, it cannot guarantee success and should not obscure consideration of other pathogenic mechanisms. Impaired insulin signaling (arising from insulin deficiency or insulin insensitivity), hypertension, and dyslipidemia may all operate individually, communally, or in combination with hyperglycemia to produce neuropathy [2]. A more sophisticated appreciation of risk factors for neuropathy in diabetic patients will hopefully lead to more careful clinical characterization of patients that can be in turn used to identify potential pathogenic mechanisms.

### *Neuropathy and Pain*

In some diabetic patients, onset of pain is attributable to acute normalization of blood sugar at the onset of insulin therapy (insulin neuritis) [3], while in others it coincides with dramatic weight loss. Aside from these particular conditions, it is frequently estimated that 10–20% of patients with diabetic neuropathy exhibit pain as one of the symptoms. This number may well be an underestimation. A recent epidemiological study reported tingling, shooting, or burning pain in 34% of over 15,000 diabetic patients surveyed in the community [4]. Prevalence of pain was greater in patients with type 2 diabetes than those with type 1 diabetes (35% vs. 22%), greater in females with diabetes than in males (38% vs. 31%), and showed variability between ethnic groups within the study cohort. These findings present a strong case that the prevalence of pain in diabetic patients is underestimated when studied in the hospital and academic setting. Nevertheless, pain was by no means a universal feature of patients with clinically demonstrable neuropathy using the neurological disability score (NDS), while 26% of diabetic patients with pain had no detectable clinical neuropathy. Such discrepancies may to some extent reflect the inadequacy of the NDS for detecting early neuropathy but the findings also highlight the lack of a clear and consistent pathogenic mechanism to link diabetes with neuropathy or neuropathy with pain.

Pain is only one manifestation of aberrant function of the nervous system in diabetic patients. Indeed, sensory loss is a more common presentation, while both pain and sensory loss can coexist in the same patient. The progressive distal neurodegeneration that is the hallmark of diabetic neuropathy adds an additional layer of complexity when attempting to identify pathogenic mechanisms for neuropathic pain in diabetic patients and nerve degeneration has been evoked as a mechanism of both pain generation and also disappearance of pain over time. Comprehensive nerve biopsy studies were unable to distinguish between painful and painless diabetic neuropathy based on large or small fiber pathology in nerve trunks and there was also no association between pain and regenerating fibers [5–7]. More recently, a potential association between nerve degeneration and pain has been revived by reports that length of small sensory fibers in the epidermis or cornea can identify diabetic patients with or without pain [8]. A report that lifestyle intervention both diminished pain and increased

epidermal small fiber density in prediabetic subjects further encourages an association between pain and distal fiber loss [9]. Assessing the most distal regions of sensory axons rather than axons in nerve trunks may offer improved assay sensitivity, and such findings have reawakened consideration of mechanisms by which nerve degeneration may cause pain. These include ectopic activity by destabilized degenerating fibers and the release of factors released from degenerating fibers activating adjacent fibers to produce ephaptic cross talk. However, these encouraging new observations must be viewed with caution because the apparent associations may be coincident rather than causal. The emerging appreciation that the peripheral terminals of small fibers in the skin express growth-associated proteins that suggest continuous plasticity [10] so that changes in nerve length need not reflect degeneration so much as remodeling is also yet to be addressed. Finally, it should be remembered that many degenerative peripheral neuropathies are painless so that there is no universal association between degenerating axons and pain.

Aside from nerve degeneration, there have also been attempts to correlate the presence of pain with other features of the diabetic condition. Acute hyperglycemia enhances pain perception in normal subjects [11], although pain in diabetic subjects is not associated with acute increases in blood glucose levels [12]. Depression, a common consequence of suffering from diabetes and its complications, can also exaggerate pain perception [13] but is more likely to confound attempts to show linearity between any physical disorder and pain intensity in diabetic subjects than serve as an initiating mechanism. Most recently, increased plasma levels of soluble ICAM, a marker of endothelial cell dysfunction, and of C-reactive protein (CRP) were found in patients with painful vs. painless diabetic neuropathy [14]. Both proteins are used as markers of general systemic inflammation, raising the possibility of a selective inflammatory component to pain. However, many other indices of inflammation did not vary between diabetic patients with and without pain and it remains to be determined whether these two specific proteins can promote neuropathic pain. In general, the broad assumptions that hyperglycemia causes neuropathy and that nerve degeneration, or subsequently frustrated regeneration, causes pain are not particularly well supported by the current clinical literature and there is no strong evidence linking specific physical or chemical features of diabetes or neuropathy with the presence or the absence of pain.

### ***Current Therapies***

In the absence of an established pathogenic mechanism for either neuropathy or pain, there is no prophylactic therapy against painful diabetic neuropathy. Current approaches are restricted to trying to alleviate established pain by working through a laundry list of drugs that are effective in other pain conditions and hoping to find one with enough efficacy and minimal side effect profile to be useful. Only duloxetine (Cymbalta) and pregabalin (Lyrica) currently have FDA-approved labeling for treating painful diabetic neuropathy. A number of excellent recent reviews have

offered practical approaches to working through the drugs that are in current clinical use to treat painful diabetic neuropathy, with anticonvulsants and antidepressants usually heading the list [15–18]. Most current interventions clearly interfere with normal sensory processing mechanisms and therefore need not necessarily offer clues to the underlying pathogenesis of pain in diabetic patients. However, it has been speculated that pain can arise from a diabetes-induced inappropriate over-expression of components of normal sensory processing mechanisms so that inhibitors of these components may suppress pain across a broader therapeutic window before disrupting normal functions. The gabapentinoids, which bind to the  $\alpha 2\delta 1$  subunit of calcium channels, and local anesthetics that block voltage-gated sodium channels may both plausibly fit into this category, although there is not yet clear evidence that either calcium or sodium channels are selectively over-expressed in diabetic patients with painful neuropathy other than an intriguing report of increased nodal persistent sodium currents in large myelinated fibers [19]. Alpha-lipoic acid (ALA) has also been reported to have some success against pain in diabetic patients [20]. While ALA is frequently presented as an antioxidant, the extrapolation that pain is related to oxidative stress should be treated with caution, as ALA also has a number of other biological actions. Recent efforts to screen nerve biopsies from diabetic patients for altered gene expression patterns that could provide biomarkers for degenerative neuropathy [21] have yet to be extended to differentiate between patients with painful and painless neuropathy. Difficulties in obtaining appropriate material from well-characterized subjects have caused many investigators to resort to performing mechanistic studies in animal models of diabetic neuropathy.

## Clues from Animal Models of Painful Diabetic Neuropathy

### *Pain in Diabetic Rodents*

Direct evidence of pain perception and magnitude cannot be obtained from animals that are unable to express themselves emotionally [22] while rodents are notoriously unreliable when using quantitative systems devised for clinical pain studies such as the visual analog scale. Diabetic rodents also do not show behavioral evidence of spontaneous pain such as altered audible or ultrasonic vocalizations [23], limb guarding, or autotomy, while reduced locomotion [24] has yet to be decisively associated with ongoing pain rather than other physiological consequences of diabetes. Investigators have therefore largely resorted to using nociceptive tests that measure behavioral or physiological responses to mechanical, thermal, and chemical stimuli as surrogate assays for pain [22]. All such assays of hyperalgesia (exaggerated responses to a stimulus that evokes a response in normal animals) and allodynia (a response to a stimulus that does not evoke a response in normal animals) carry caveats that include the potential involvement of altered stimulus transduction properties of the skin and of motor and/or effector dysfunction in any modified response. Nevertheless, there is now extensive literature using such tests in diabetic rodents to investigate potential mechanisms of pain and to evaluate efficacy of new therapies.

Behavioral responses to mechanical and thermal stimuli are the most widely used assays in animal studies of painful diabetic neuropathy. As noted above, representing these assays as measuring neuropathic pain is perhaps overly optimistic, but some of these tests do have clinical correlates. Thus, the withdrawal of the paw from von Frey filaments of low force generation (1–15 g) can reasonably be described as allodynia [25] and has a parallel in the tactile allodynia reported by diabetic patients in response to the same von Frey filaments upon clinical examination or contact with clothing or bedding in daily life. Similarly, paw withdrawal from a heat stimulus can be equated to the measurement of thermal pain perception thresholds used in quantitative sensory testing procedures. Other tests commonly employed in animal studies such as paw withdrawal from escalating pressure forces in the 50–300 g range and those that stimulate the tail with mechanical or thermal stimuli are perhaps further removed from clinical equivalents. Diabetic rats, but not mice, also exhibit increased paw flinching responses to local injection of formalin [25]. While there is clearly no clinical correlate for this test, it offers the opportunity to study both the acute response to peripheral tissue injury and a delayed response that is driven by spinal sensitization mechanisms rather than increased peripheral nerve activity.

Allodynia to von Frey filaments is an early and constant feature in rat models of both type 1 [25] and type 2 diabetes [26], with impaired insulin signaling contributing to the pathogenic mechanism, independent of hyperglycemia [27]. Whether diabetic mice also show allodynia or loss of responsiveness to the filaments appears to vary between investigators, assay system, mouse strains, and models of diabetes. Reports of paw responses to heat in diabetic rodents are also somewhat variable. In type 1 and type 2 diabetic rats, early thermal hyperalgesia can be stable over time or progress to hypoalgesia [28, 29]. The retention of hyperalgesia appears to be a feature of rats with residual endogenous insulin or receiving insulin supplementation [30], whereas progression to thermal hypoalgesia is accompanied by depletion of heat-sensitive epidermal fibers [31]. Both early thermal hyperalgesia and subsequent thermal hypoalgesia can be prevented by blocking glucose metabolism by aldose reductase [29]. It therefore appears that hyperalgesia is driven by hyperglycemia whereas the progression to hypoalgesia requires both hyperglycemia and marked insulin deficiency. By contrast, diabetic mice progress rapidly towards a thermal hypoalgesia that precedes epidermal fiber depletion [32]. Taken together, rats appear to be a more reliable model for studying mechanisms of diabetes-induced pain than mice, with tactile allodynia being a particularly useful feature that is widely reported, stable over many months and has a clear clinical correlate.

### ***Mechanisms and Therapies Predicted from Diabetic Rodents***

Investigations of mechanisms underlying altered sensory responses in diabetic rodents frequently search for altered expression of molecules that may influence sensory functions. This may involve a novel gain of function, increased expression or release of molecules that might amplify sensory processing, or reduced expression/release of molecules that inhibit sensory processing. A parallel approach is to evaluate efficacy of

potential therapeutics and use their presumed mode of action to deduce putative pathogenic mechanisms. The location of the generator and/or amplifier sites of enhanced nociception in diabetic rodents remains controversial and many sites may coexist, possibly reflecting the variable human condition described above.

## Peripheral Drive

The perception of pain by diabetic patients as being predominantly bilateral and most intense in the hands and feet supports the reasonable and widely held assumption that systemic injury leading to activation of primary sensory neurons underlies painful diabetic neuropathy. In diabetic rats, tactile allodynia is also bilateral and localized to the hind paws, but not the flank.

*Neurodegeneration.* The potential for ephaptic cross talk driven by degenerating fibers in nerve trunks that has emerged from biopsy studies in diabetic patients (see above) is unlikely to be replicated in animal models of diabetes. There is no overt nerve fiber degeneration in nerve trunks of short-term diabetic rats or mice while depletion of intra-epidermal nerve fibers appears well after the onset of tactile allodynia and thermal hyperalgesia in STZ-diabetic rats [25, 31]. STZ-diabetic mice show more rapid epidermal fiber depletion [32] but are not reliable models of allodynia or hyperalgesia. In the absence of any clear association between overt nerve pathology and indices of painful neuropathy in diabetic rodents, interest has largely focused on cellular and molecular changes in primary afferents of diabetic rodents that could either enhance sensitivity of nociceptors to stimuli or exaggerate activity once stimulated.

*Nociceptor activation.* Some animal models of neuropathic pain exhibit increased local levels of pro-inflammatory molecules that can sensitize primary afferent terminals and intraplantar injection of protein kinase C inhibitors can suppress nociceptor activity and behavioral indices of pain in diabetic rats [33], implying ongoing local stimulation. However, diabetes tends to suppress inflammatory responses, there being an impaired skin allergic inflammatory response, reduced mast cell numbers, reduced histamine release, and reduced levels of TNF $\alpha$  and IL-1 $\beta$  in skin of diabetic rodents [34, 35]. There are also contradictory reports of both increased [36, 37] and decreased [38, 39] levels of mRNA and protein for NGF, a neurotrophic factor associated with pain. In the absence of clear evidence of a local inflammatory state that could activate nociceptors, the possibility that peripheral sensitization could also develop as a result of altered expression and/or activity of receptors that transduce sensory stimuli has begun to be explored. There is abnormal expression of the heat-sensitive TRPV1 channel in cell bodies of large sensory neurons that could make such fibers heat sensitive [40, 41]. Moreover, suppression of the mechanosensitive TRPV4 receptor attenuated hyperalgesia to deep mechanical pressure [42] while intraplantar injection of antagonists of the chemosensitive TRPA1 channel alleviated tactile allodynia [43, 44]. What aspect of the diabetic condition triggers altered expression or activity of these transducers of sensory stimuli remains to be established, although in the case of TRPA1, there is some evidence that hyperglycemia-driven methylglyoxal formation may be involved [45].

*Nociceptor activity.* The electrical properties of peripheral nociceptors can be altered by changes in expression of axonal ion channels involved in action potential formation, shaping, and patterning, while ion channels located on the central terminals of primary afferents dictate whether electrical activity of primary afferents translates into neurotransmitter release. Increased expression of voltage-gated ion channels has been implicated in spontaneous activity of nociceptors and exaggerated electrophysiological and behavioral responses to stimuli in a number of models of neuropathic pain arising from nerve trauma. In diabetic animals, spontaneous activity of sensory neurons has been reported in some [46, 47], but not all [48], studies and altered firing patterns after C fiber stimulation may occur in subpopulations of fibers [49, 50]. Primary afferent instability has been attributed to altered expression, membrane insertion, and function of voltage-gated sodium and potassium channels, although there is some inconsistency in reports as to which channels and subunits are affected in nerve of diabetic rodents [51–55]. Increased protein levels of the  $\alpha 2\delta 1$  subunit of the calcium channel [56] and mRNA for L-type [57, 58] calcium channels have also been detected in the DRG of diabetic rats, and there are increased calcium currents through N-type [59] and T-type [60] calcium channels in sensory neurons from such animals. The efficacy of low systemic doses of sodium channel blockers such as lidocaine [25], spinal delivery of N-type (but not L type) calcium channel blockers [61], gene silencing of the T-type calcium channel [62], and gabapentinoids that bind the  $\alpha 2\delta 1$  subunit of calcium channels [56, 63] in alleviating tactile allodynia of diabetic rodents all support the idea that increased expression of voltage-gated ion channels contributes to allodynia and offers a broader therapeutic window for ion channel blockers to operate before interfering with normal sensory function. An increased membrane density of voltage-gated sodium and calcium channels also provides an appealing mechanism to explain the clinical efficacy of transdermal lidocaine and gabapentinoids in alleviating pain in some patients with diabetic neuropathy [15–18], while similar reports of the efficacy of ALA [20] could reflect the ability of this compound to inhibit T-type calcium channels [64]. What aspect of diabetes causes the neuron to increase the expression of these proteins is not yet known.

## Spinal Amplification

Although most attention has focused on diabetes-induced peripheral nerve damage, there is a long history of autopsy studies demonstrating that the spinal cord of diabetic patients also undergoes degeneration of both white and gray matter [65]. Magnetic resonance imaging (MRI) studies have confirmed early spinal cord damage in live patients [66] and highlight the potential for disruption of spinal sensory processing to contribute to pain in diabetic patients. There is currently little firm evidence available from clinical studies to support or refute this speculation, and attempts to use constituents of the cerebrospinal fluid to segregate patients with painful and painless diabetic neuropathy have not yet been fruitful. There is, however, a growing literature suggesting that spinal sensory processing is abnormal in diabetic rodents and may contribute to indices of painful neuropathy via a variety of mechanisms. This is particularly appealing,



as studies in diabetic rats using functional MRI and spinal microdialysis of evoked excitatory neurotransmitter release have indicated that primary afferent excitatory input to the cord is paradoxically diminished following peripheral stimulation, despite enhanced behavioral responses [67–69]. Impaired excitatory input may negate increased sensitivity of peripheral nerves themselves so that diminished input may require signal amplification during spinal or supraspinal processing.

*Spinal Sensitization.* It is becoming increasingly recognized that while classical cell-mediated inflammation is a dangerous and extreme condition in the CNS, a form of inflammation directed by endogenous glial cells accompanies many diseases of the nervous system, including pain states [70]. Diabetes has impact on all glial cells of the spinal cord and the activation of oligodendrocytes, astrocytes, and microglia has the potential to drive spinal sensitization mechanisms that amplify sensory input and offer potential sites for therapeutic intervention.

There is a report of spontaneous activity in postsynaptic dorsal horn neurons of diabetic rats [71] and direct delivery of substance P to the spinal cord elicits an enhanced behavioral response in such animals [72]. Increased paw flinching during phase 2, but not phase 1, of the formalin test in diabetic rats also implies that a spinally mediated hyperalgesia has developed [25]. Release of pro-inflammatory prostaglandin E in the spinal cord after peripheral stimulation is increased and prolonged in diabetic rats [73], despite reduced primary afferent input. These data suggest that diabetes induces a state of spinal sensitization in rats that amplifies peripheral input, even when release of excitatory neurotransmitters is attenuated. The pathogenesis of spinal sensitization revealed during the formalin test involves glucose metabolism by aldose reductase in spinal oligodendrocytes, which leads to over-expression of the prostaglandin-forming enzyme cyclooxygenase-2 (COX-2) [74]. Inhibitors of aldose reductase and COX-2, applied either directly to the spinal cord or given systemically in formulations that cross the blood:brain barrier, attenuate diabetes-induced hyperalgesia [73, 74]. Occasional reports that aldose reductase inhibitors can ameliorate painful diabetic neuropathy [75, 76] and the widespread nonprescription use of NSAIDs by diabetic patients with pain may validate the relevance of this mechanism to the human condition, although difficulties in safely delivering sufficient quantities of either drug to the spinal cord may hamper clinical efficacy.

Aside from oligodendrocytes, diabetes also impacts other spinal glia. The growing interest in spinal microglia as mediators of spinal sensitization following nerve injury has been extended to diabetes with reports that microglia showing morphological changes suggestive of activation in the spinal cord of diabetic rodents and that inhibitors of signaling pathways associated with microglial activation can ameliorate behavioral indices of allodynia and hyperalgesia in such animals [77–79]. Cannabinoids [80], bradykinin B1 receptor antagonists [81], and other drugs that intervene against microglial activation may therefore have therapeutic potential, again subject to delivery issues. The role of astrocytes in regulating their local microenvironment and their proposed involvement in other forms of neuropathic pain [70] makes them an equally intriguing area of study, and reduced expression of GFAP [82], usually an activation marker, in spinal astrocytes of diabetic rats offers the potential for impaired neurotransmitter clearance [83].



*Disinhibition.* Sensory processing in the spinal cord undergoes tonic inhibition via descending and local control systems that can also be adjusted in response to sensory input. Disruption of these inhibitory systems can lead to enhanced sensory output from the cord in a process termed disinhibition that has been linked to assorted neuropathic pain states [84]. Both basal and stimulus-evoked release of the inhibitory neurotransmitter GABA [68] is increased in the spinal cord of STZ-diabetic rats, a finding that initially appears incompatible with increased behavioral indices of pain sensation in these animals, unless it is a response to impaired GABA receptor expression or function. However, pharmacological studies suggest that the inhibitory function of GABA<sub>A</sub> receptors is diminished in the spinal cord of STZ-diabetic rats [85] and that it converts to an excitatory receptor due to shifts in the chloride equilibrium potential mediated by depletion of the potassium chloride co-transporter KCC2 [85, 86]. This parallels some nerve injury models of neuropathic pain that also show reduced spinal KCC2 expression and excitatory GABA function [87]. A therapeutic application of this mechanism is that GABA<sub>A</sub> antagonists alleviate allodynia and hyperalgesia in diabetic rats [85], although this counterintuitive therapy has yet to be explored clinically. The pathogenesis of increased GABA release and reduced KCC2 expression in the spinal cord of diabetic rats remains to be established. Other spinal inhibitory systems, such as the descending serotonin system, appear to operate normally in diabetic rats, and activation of spinal 5HT<sub>2A</sub> receptors may underlie the pain-relieving effects of serotonin and serotonin:noradrenaline reuptake inhibitors such as duloxetine in diabetic rodents and patients [88].

## Higher CNS Involvement

Autopsies of brains from diabetic patients demonstrate widespread encephalopathy, and impaired insulin signaling has been linked with cognitive impairments that resemble early Alzheimer's disease in both humans and animals [89, 90]. A role for CNS dysfunction in painful neuropathy is also suggested by recent MRI studies that identified differences in blood flow, neuronal metabolism [91], and activity in the thalamus of diabetic patients with or without painful neuropathy [92–96]. Diabetic rats also show increased neuronal activity, as measured by glucose uptake, in brain regions that discriminate pain [97], while a preliminary study suggested that the thalamus could act as a generator site of spontaneous activity in diabetic rats [98]. Whether these observations reveal painful diabetic neuropathy to have a central origin remains to be determined, although there is clinical precedence for the onset of diabetes inducing phantom limb pain [99].

## Summary

Studies in diabetic rodents have identified numerous molecular, cellular, and physiologic disorders that may contribute to behavioral indices of enhanced nociception. Perhaps the strongest argument supporting the validity of diabetic rodents to model

painful diabetic neuropathy is that drugs effective in diabetic patients such as the gabapentinoids, duloxetine, tricyclic antidepressants, and lidocaine also show clear efficacy in the animal models. However, all of these drugs have been applied to patients after serendipitous clinical observations rather than emerging from targeted preclinical drug development programs. The disturbingly large number of diverse agents that also alleviate established allodynia or hyperalgesia in diabetic rodents offers some hope that new therapies may take the desired route from bench to bedside, or perhaps may indicate an unfortunate tendency for identifying false positives. Less attention has been paid to prophylactic therapies and, other than the role of glucose metabolism by aldose reductase in spinal sensitization, pathogenic mechanisms linking impaired insulin signaling and/or hyperglycemia with the proposed molecular and cellular underpinnings of allodynia and hyperalgesia largely remain to be explored.

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