Diabetic Painful and Insensate Neuropathy: Pathogenesis and Potential Treatments

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Summary: Advanced peripheral diabetic neuropathy (PDN) is associated with elevated vibration and thermal perception thresholds that progress to sensory loss and degeneration of all fiber types in peripheral nerve. A considerable proportion of diabetic patients also describe abnormal sensations such as paresthesias, allodynia, hyperalgesia, and spontaneous pain. One or several manifestations of abnormal sensation and pain are described in all the diabetic rat and mouse models studied so far (i.e., streptozotocin-diabetic rats and mice, type 1 insulinopenic BB/Wor and type 2 hyperinsulinemic diabetic BBZDR/Wor rats, Zucker diabetic fatty rats, and nonobese diabetic, Akita, leptin- and leptin-receptor-deficient, and high-fat diet-fed mice). Such manifestations are 1) thermal hyperalgesia, an equivalent of a clinical phenomenon described in early PDN; 2) thermal hypoalgesia, typically present in advanced PDN; 3) mechanical hyperalgesia, an equivalent of pain on pressure in early PDN; 4) mechanical hypoalgesia, an equivalent to the loss of sensitivity to mechanical noxious stimuli in advanced PDN; 5) tactile allodynia, a painful perception of a

light touch; and 5) formalin-induced hyperalgesia. Rats with short-term diabetes develop painful neuropathy, whereas those with longer-term diabetes and diabetic mice typically display manifestations of both painful and insensate neuropathy, or insensate neuropathy only. Animal studies using pharmacological and genetic approaches revealed important roles of increased aldose reductase, protein kinase C, and poly(ADP-ribose) polymerase activities, advanced glycation end-products and their receptors, oxidative-nitrosative stress, growth factor imbalances, and C-peptide deficiency in both painful and insensate neuropathy. This review describes recent achievements in studying the pathogenesis of diabetic neuropathic pain and sensory disorders in diabetic animal models and developing potential pathogenetic treatments. **Key Words:** Animal models, diabetic insensate neuropathy, diabetic painful neuropathy, formalin-induced hyperalgesia, mechanical hyper- and hypoalgesia, pathogenetic treatments of diabetic neuropathic pain and sensory loss, symptomatic treatments of diabetic pain, tactile allodynia, thermal hyper- and hypoalgesia.

DIABETIC PAIN AND SENSORY LOSS: PREVALENCE, CURRENT TREATMENT OPTIONS, AND LIMITATIONS OF EXISTING ANIMAL MODELS

Diabetic distal symmetric sensorimotor polyneuropathy, typically referred to as peripheral diabetic neuropathy (PDN), affects at least 50% of diabetic patients in the United States, and is the leading cause of foot amputation.^{1,2} Advanced PDN is associated with elevated vibration and thermal perception thresholds that progress to sensory loss, occurring in conjunction with degeneration of all fiber types in the peripheral nerve. A proportion of patients with PDN also describe abnormal sensations such as paresthesias, allodynia, hyperalgesia, and

spontaneous pain that sometimes seriously affect their quality of life.³ The prevalence of pain varies from 10% to 20% in subjects with diabetes mellitus and from 40% to 50% in those with diabetic neuropathy.⁴ As A.I. Vinik put it, "Pain in neuropathy is multifactorial and can occur at different levels, starting at the peripheral sympathetic nervous system in the skin (C fibers) and migrating to involve A β and A δ fibers to produce allodynia, continuing with reorganization at the cord-level, cold allodynia, and finally, within the central nervous system, spontaneously as opposed to provoked pain."⁵ The natural course of painful diabetic neuropathy is variable, with many patients experiencing spontaneous improvement and resolution of pain.⁴ Except for two pathogenetic drugs (i.e., the aldose reductase inhibitor epalrestat, approved in Japan, and the antioxidant lipoic acid, approved in several countries), current therapeutic options are limited to symptomatic treatment with tricyclic compounds, selective serotonin noradrenaline reuptake inhibitors, anticon-

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vulsants, opiates, capsaicin, and membrane stabilizers.⁶ Several new symptomatic drugs (i.e., the serotonin and norepinephrine reuptake inhibitor duloxetine and two anticonvulsants, pregabalin and gabapentin) have recently been approved for treatment of diabetic neuropathic pain in the United States; however, their use is often associated with problems such as insufficient efficacy, development of adverse side effects, and cost effectiveness. The development of better treatment modalities for diabetic neuropathic pain and sensory loss should be based on pathogenetic mechanisms.

Pathogenesis of diabetic painful neuropathy is poorly studied, although identification of impaired cutaneous endothelium-related vasodilation and C-fiber-mediated vasoconstriction as well as increased sural nerve epineurial blood flow in diabetic subjects with painful, compared to those with painless, neuropathy suggests the importance of hemodynamic factors.^{7,8} A recent study with thalamic proton magnetic resonance spectroscopy implicates thalamic neuronal dysfunction.⁹

Understanding the biochemical mechanisms underlying diabetic neuropathic pain and sensory disorders requires preclinical studies in animal models. Existing animal models of diabetic painful and insensate neuropathy, however, have serious limitations.³ Diabetic rats and mice have limited life span and rarely show evidence of overt neuropathy, such as demyelination, axonal degeneration, fiber loss, or axonal regeneration in their peripheral nerves. This makes diabetic rodents unsuitable for studying the contribution of these phenomena of advanced PDN to pain or to loss of sensory function. In addition, it is not possible to quantify spontaneous pain in animals. Nonetheless, despite these limitations, assessment of behavioral responses to external stimuli in diabetic rats and mice (i.e., thermal and mechanical hyper- and hypoalgesia, tactile allodynia, as well as formalin-induced spontaneous nociceptive behavior) has led to identification of a number of mechanisms of abnormal sensation and pain in diabetes. This review summarizes current knowledge on the mechanisms of diabetic painful and insensate neuropathy obtained through the studies in diabetic animal models and describes pathogenetic treatments for diabetic neuropathic pain and sensory loss emerging from experimental studies and clinical trials.

PATHOGENESIS AND EXPERIMENTAL TREATMENTS OF DIABETES-ASSOCIATED THERMAL HYPER- AND HYPOALGESIA

A considerable proportion of human subjects with PDN display altered cutaneous sensory perception including warm and cold thermal perception, vibration, touch-pressure sensation, and current perception, and vibratory and thermal testing is typically performed during primary screening for diabetic neuropathy and in clinical

trials of new therapeutics.^{10,11} Assessment of thermal sensitivity in diabetic rats and mice is performed using a number of behavioral tests, among which paw withdrawal, tail flick, and hot-plate tests are the most widely used.

The tail flick test, which does not have an anatomical equivalent in humans, measures the latency for tail movement from an escalating radiant heat source and reflects activity of a simple spinal reflex arc, thus providing information on peripheral nerve and spinal function in isolation from higher nociceptive processing and cognitive systems. The Hargreaves method, with escalating radiant heat stimulation applied to the plantar surface of a single paw, measures the latency of the paw withdrawal from a noxious stimulus source, and includes supraspinal sensory processing. The hot-plate test provides rapid stimulation of all paws, tail, and in some cases—as when used in obese leptin-deficient (*ob/ob*), leptin receptor-deficient (*db/db*), and high-fat diet-fed mice—parts of abdominal skin. The tail-flick and hot-plate tests are less sensitive in detecting thermal hyper- or hypoalgesia and effects of experimental therapies in diabetic rats and mice.^{12–14} In general, the hot-plate test is seldom used in diabetic rat studies, although it has been sensitive enough to demonstrate thermal hyperalgesia in several studies.^{15,16}

Thermal hyperalgesia has been registered in some patients with mild PDN, whereas advanced-stage disease is typically characterized by elevated thermal perception thresholds (hypoalgesia) that progress to sensory loss, occurring in conjunction with unmyelinated peripheral nerve fiber degeneration.^{17,18} Thus, studies of mechanisms underlying both thermal hyperalgesia and thermal hypoalgesia and the development of agents to prevent and treat both disorders are clinically relevant.

Thermal hyperalgesia has been described in streptozotocin (STZ)-diabetic and Zucker diabetic fatty rats with short-term (2–8 weeks) diabetes,^{19–22} as well as in type 1 insulinopenic BB/Wor and type 2 hyperinsulinemic diabetic BBZDR/Wor rats.^{23,24} This phenomenon develops with participation of multiple pathogenetic mechanisms, including (but not limited to) increased aldose reductase (AR),¹⁹ the advanced glycation end product/receptor for advanced glycation end product (AGE/RAGE) axis,²⁵ protein kinase C (PKC),²⁶ poly(ADP-ribose) polymerase (PARP),¹² and angiotensin converting enzyme (ACE) activities,²² oxidative stress,^{20,21} and C-peptide deficiency.²⁷ Thermal hyperalgesia in STZ-diabetic rats was prevented or reversed by the AR inhibitor lidorestat,¹⁹ the AGE formation inhibitor pyridoxamine,²⁵ the PKC LY333531,²⁶ several antioxidants (including α -lipoic acid,²⁰ the hydroxyl radical scavenger dimethylthiourea,²¹ the xanthine oxidase inhibitor allopurinol,²⁸ and taurine²⁹), the PARP inhibitors 1,5-isoquinolinediol¹² and nicotinamide,³⁰ and the neurocytokine interleukin-

6,³¹ as well as by transgene-mediated expression of enkephalin in dorsal root ganglia neurons.³² C-peptide and the glutamate carboxypeptidase II inhibitor treatments alleviated thermal hyperalgesia in type 1 diabetic BB/Wor rats,^{27,33} and taurine in type 2 Zucker diabetic fatty rats.³⁴

Whereas mechanisms underlying analgesic effect of these agents require further studies, a number of the treatments mentioned here have been reported to counteract glutamate excitotoxicity by reducing glutamate accumulation,^{33,35,36} to restore impaired Ca^{2+} homeostasis and signaling,³⁷ to inhibit mitogen-activated protein kinase (MAPK) activation,³⁸ and to counteract production of proinflammatory cytokines.³⁹ All these biochemical changes are involved in generation of pain.^{40–43} Enkephalin has been shown to activate the presynaptic δ -opioid receptor and, through inhibition of PKC and p38 MAPK, to prevent the increase in the neuronal $\text{Na}_v1.7$ tetrodotoxin-sensitive voltage-gated sodium channel isoform (which plays a critical role in nociception) in dorsal root ganglia.³²

Thermal hypoalgesia is present in a large proportion of patients with advanced PDN. This condition has been described in STZ-diabetic rats with longer-term (≥ 12 weeks) diabetes,^{19,44} and in a number of diabetic mouse models, including SVEV129 $\times$ C57BL/6,⁴⁵ C57BL/6J,^{13,14,46,47} Swiss Webster,^{48–50} 129S1/SvImJ,⁴⁴ and CD1⁵¹ mice made diabetic with STZ, as well as non-obese diabetic (NOD) mice,⁵² leptin-deficient (*ob/ob*) mice,^{53,54} and diabetic Akita mice (V.R. Drel and I.G. Obrosova, unpublished data), as well as in two animal models of prediabetes and obesity, Zucker fatty rats⁵⁵ and high-fat diet-fed C57BL/6J mice.⁵⁶ Data for leptin receptor-deficient (*db/db*) mice^{57,58} and Zucker diabetic fatty rats^{59,60} are contradictory, and both normal and reduced thermal sensation in these two animal models have been reported.

Surprisingly, a number of mechanisms contributing to increased thermal sensitivity in the rat models with relatively short-term duration of diabetes were also implicated in development of thermal hypoalgesia in rats with diabetes of longer duration or diabetic mice. Those include increased AR activity,¹⁹ activation of the AGE/RAGE axis,^{25,45,48} and oxidative-nitrosative stress,^{13,14,47,53} as well as activation of ACE^{22,61} and PARP.^{12,44} Several studies, including one identifying an important role of insulin signaling,⁵¹ implicate neurotrophic factor deficiency in diabetes-induced thermal sensory loss. Correspondingly, a number of pharmacological agents and neurotrophic factors showed efficacy against this disorder. Thermal hypoalgesia in STZ-diabetic rats and *ob/ob* mice was prevented or alleviated by AR inhibitors.^{19,53} A number of pharmacological agents increased or normalized thermal response latencies in STZ-diabetic rats and mice: peroxynitrite decomposition catalysts,^{13,46} the PARP inhibitor

10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diazabenz[de]anthracen-3-one (GPI-15427),⁴⁴ and the 12/15-lipoxygenase inhibitors cinnamyl-3,4-dihydroxy- α -cyano-cinnamate⁶² and baicalein, as well as Schwann cell-derived ciliary neurotrophic factor,¹⁹ TX14(A) (a neurotrophic peptide deriving from prosaposin),⁶³ and a sonic hedgehog-IgG fusion protein.⁶⁴ A prolonged expression of neurotrophin-3 in mice, achieved by herpes simplex virus (HSV)-mediated gene transfer with gene expression under the control of an HSV latency promoter, provided protection against diabetes-associated thermal hypoalgesia over a 6-month period.⁵⁰ A peroxynitrite decomposition catalyst treatment also counteracted thermal hypoalgesia in the *ob/ob* mouse model.⁴⁷ An ACE inhibitor (enalapril) and a hydroxymethylglutaryl-CoA reductase inhibitor (rosuvastatin) alleviated thermal sensory loss in Zucker fatty rats.⁶¹ The vasoepitidase (i.e., ACE and neutral endopeptidase) inhibitor AVE7688 improved thermal nociception in Zucker diabetic fatty rats.⁵⁹ Finally, thermal hypoalgesia associated with alimentary obesity and glucose intolerance can be cured by switching from high-fat to normal-fat diet.⁵⁶ Thus, both dietary and pharmacological approaches should be developed for treatment of this disorder in human subjects with diabetes mellitus.

Until recently, for lack of objective measures, small-caliber nerve fiber degeneration has not been evaluated in either human subjects with diabetes mellitus or animal models. Emerging new techniques such as corneal confocal microscopy, a reiterative, rapid, noninvasive *in vivo* imaging technique for quantitative assessment of degeneration and regeneration of corneal nerve fibers,^{65,66} and skin biopsy with visualization and quantitation of epidermal nerve fibers^{67–69} have generated enormous potential for a better understanding the mechanisms underlying small nerve fiber degeneration, a phenomenon that may ultimately lead to a complete loss of sensory function and that is a major cause of foot amputation.

Clinical studies revealed that subjects with both type 1 and type 2 diabetes exhibit epidermal nerve fiber loss.^{67–69} In diabetic patients, distal leg intraepidermal nerve fiber density showed significant negative correlations with warm and cold thermal threshold, heat pain, pressure sense, neurological disability score, total sensory score, and total neuropathy score.⁶⁸ In another study, intraepidermal nerve fiber density, branch density, and branch length showed a progressive reduction with increasing severity of neuropathy, which was significant in diabetic patients with mild, moderate, and severe neuropathy.⁶⁶

Corneal confocal microscopy showed a progressive reduction in corneal nerve fiber density and branch density, but the latter was significantly reduced even in diabetic patients without neuropathy.⁶⁶ Both intraepidermal and corneal nerve fiber densities correlated signifi-

cantly with cold detection and heat as pain thresholds. Intraepidermal and corneal nerve fiber lengths were reduced in patients with painful, compared with painless, diabetic neuropathy. Whereas studies of corneal nerve fibers have never been performed in animal models of diabetes, evidence for reduced intraepidermal nerve fiber density in a number of diabetic animal models in emerging. In particular, this phenomenon was described in STZ-diabetic⁴⁴ and Zucker diabetic fatty rats⁶⁰ and in C57Bl/6J,^{13,14,46,47,70} Swiss Webster,^{48,49} 129S1/SvImJ,⁴⁴ C57B6:129S7,⁷¹ and CD1⁵¹ mice made diabetic with STZ, as well as in leptin-deficient (*ob/ob*)^{53,54} and leptin receptor-deficient (*db/db*) mice.⁵⁷ Note that high-fat diet-fed mice with impaired glucose tolerance and obesity, but without overt hyperglycemia, do not display any intraepidermal fiber loss,⁵⁶ similarly to human subjects with metabolic syndrome.⁷²

Is intraepidermal nerve fiber density a reliable morphological correlate of thermal allodynia in diabetic animal models? From a neurotherapeutic discovery perspective, should a candidate drug for treatment of abnormal thermal sensation associated with PDN be necessarily effective on physiological measures of both thermal sensitivity and intraepidermal nerve fiber density? Taking into consideration that small sensory nerve fiber loss may be a difficult condition to reverse, especially in human subjects with advanced diabetic neuropathy, is it possible to, at least partially, correct diabetes-induced thermal hypoalgesia by agents that fail to induce small sensory nerve fiber regeneration? An answer to several of these questions has been obtained in recent animal studies. Thermal hypoalgesia was shown to develop prior to small sensory nerve fiber degeneration in STZ-diabetic C57Bl/6J mice,⁴⁶ an observation later reproduced in the STZ-diabetic Swiss Webster mouse model.⁴⁹ Zucker diabetic fatty rats displayed intraepidermal nerve fiber loss in the absence of thermal hypoalgesia.⁶⁰ Furthermore, a peroxynitrite decomposition catalyst treatment alleviated thermal hypoalgesia, but not intraepidermal nerve fiber loss, in *ob/ob* mice.⁵⁴ In another study, STZ-diabetic 12/15-lipoxygenase-deficient mice exhibited less severe thermal and mechanical hypoalgesia than did STZ-diabetic wild-type (C57Bl/6J) mice, whereas diabetes-related reduction in intraepidermal nerve fiber density was similar in both groups.⁷³ All these findings suggest that 1) intraepidermal nerve fiber loss is not an exactly reliable correlate of thermal hypoalgesia in diabetic animal models; 2) the process of drug discovery for diabetic sensory neuropathy should not heavily rely on intraepidermal nerve fiber density as a measure of therapeutic efficacy of candidate agents; and 3) pharmacological studies related to diabetic abnormal sensation and pain should not necessarily include intraepidermal nerve fiber density as an endpoint.

PATHOGENESIS AND EXPERIMENTAL TREATMENTS: DIABETES-ASSOCIATED MECHANICAL HYPER- AND HYPOALGESIA

Mechanical hyperalgesia and hypoalgesia have both been reported in human subjects with diabetes mellitus. In a recent study, mechanical hyperalgesia (pain on pressure) has been identified in 71% of human subjects with early PDN.⁷⁴ In contrast, patients with advanced PDN typically have elevated vibration perception thresholds indicative of sensory loss, a condition ultimately responsible for foot ulceration and amputation.^{75,76} Thus, studies of mechanical hyper- and hypoalgesia and development of agents to prevent and treat both disorders have clinical relevance. In experimental studies, mechanical withdrawal thresholds in diabetic rat and mouse models are assessed by paw (rats) and tail (mouse) pressure Randall–Selitto test or with a von Frey aesthesiometer and rigid von Frey filaments. Mechanical hyperalgesia has been reported in both STZ-diabetic^{20,21,30,77,78} and Zucker diabetic fatty rats,^{34,79} in which pressure pain preceded development of type 2 diabetes. In contrast, STZ-diabetic C57Bl/6J mice^{13,14,46,47} displayed elevated mechanical withdrawal thresholds. Several hyperglycemia-related mechanisms, i.e., increased AR activity,⁷⁷ oxidative-nitrosative stress,⁷⁸ and PARP activation,^{12,44} as well as impaired insulin signaling⁷⁹ have been implicated in pain on pressure in diabetic and prediabetic rat models. Correspondingly, diabetes-induced mechanical hyperalgesia in STZ-diabetic rats was corrected or alleviated by AR inhibitors,^{77,80} by antioxidants (α -lipoic acid,²⁰ dimethylthiourea,²¹ allopurinol,²⁹ and taurine³⁰), and by a peroxynitrite decomposition catalyst,⁷⁸ as well as by PARP inhibitors,^{12,44} PARP inhibitor-containing combination therapies,²² and the Na⁺/H⁺-exchanger-1 inhibitor cariporide.⁸¹ Gabapentin (an anticonvulsant agent) and insulin strongly elevated the nociceptive threshold in STZ-diabetic rats, but gabapentin elevated it above normal levels.⁸² In the same study, OT-7100 (5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5- α]pyrimidine) counteracted mechanical hyperalgesia in a dose-dependent manner. Rosuvastatin,⁸³ the PKC inhibitor LY333531,²⁶ and interleukin-6,³¹ which corrected other manifestations of peripheral diabetic neuropathy, including thermal hyperalgesia, did not reduce an exaggerated sensitivity to mechanical noxious stimuli. Notably, two other PKC inhibitors, staurosporine and protein kinase C pseudosubstrate inhibitor peptide [PKC(19-36)], elevated mechanical nociceptive thresholds in STZ-diabetic rats in a dose-dependent manner without altering nociceptive threshold in control rats.⁸⁴ Taurine also alleviated mechanical hyperalgesia in Zucker diabetic fatty rats.³⁴ Several agents, including peroxynitrite decomposition catalysts^{13,46} and 12/15-lipoxygenase inhibitors,⁶² alleviated mechanical hypoalgesia in STZ-diabetic mice.

PARP gene deficiency was associated with the absence of mechanical hypoalgesia that was clearly manifest in the corresponding wild-type STZ-diabetic mice,⁴⁴ and 12/15-lipoxygenase gene deficiency was partially protective in the same mouse model.⁷³

DIABETES-ASSOCIATED TACTILE ALLODYNIA

In addition to spontaneous pain, painful diabetic neuropathy in human subjects is also characterized by the development of tactile allodynia, a condition in which light touch is perceived as painful. Tactile allodynia is observed in a considerable proportion (30%–47%) of human subjects with diabetes mellitus.^{10,85} A similar phenomenon is observed in STZ-diabetic rats, in which the light touch (<15 g) of von Frey filaments or the light stroking of a paw induces a withdrawal response from the stimulus.⁸⁶ In diabetic mouse models, the findings on tactile sensitivity are contradictory. One research group found tactile allodynia in STZ-diabetic C57Bl/6J and 129S1/SvImJ mice, and diabetic Akita mice, as well in as *ob/ob* mice (a model of type 2 diabetes) and high-fat diet-fed mice (a model of prediabetes and alimentary obesity).^{13,14,44,46,47,53,54,56} Tactile allodynia has also been described in STZ-diabetic B6129PF2/J87 and CD1 mice.^{51,87} Others found reduced tactile sensitivity, rather than tactile allodynia, in STZ-diabetic C57Bl/6J mice and *db/db* mice.^{58,70} Tactile allodynia also develops in nerve injury models of neuropathic pain^{88–90} (e.g., in the model of sciatic nerve ischemia⁹⁰).

The mechanisms underlying diabetes-induced tactile allodynia have not been studied in detail, although considerable progress has been made in experimental studies performed in the last several years. Tactile allodynia associated with early PDN in STZ-diabetic rats was alleviated by peroxynitrite decomposition catalyst⁷⁸ and PARP inhibitor^{12,44} treatments, which suggests the involvement of nitrosative stress and PARP activation. The latter is consistent with less severe or absent tactile allodynia in STZ-diabetic inducible nitric oxide synthase-deficient and PARP-deficient mice, compared with the corresponding wild-type mice with STZ-diabetes of similar severity and duration.^{14,44} Other agents effective against tactile allodynia in STZ-diabetic rat model include nitecapone, a catechol-*O*-methyltransferase inhibitor with potent antioxidant properties,⁹¹ agmatine, an endogenous cationic amine resulting from the decarboxylation of L-arginine,⁹² allopurinol,²⁸ interleukin-6,³¹ melatonin,⁹³ benfotiamine,⁹⁴ and the cannabinoid agonist WIN 55,212-2,⁹⁵ as well as a cocktail of B₁, B₆, and B₁₂ vitamins.⁹⁶ Tactile allodynia in STZ-diabetic mice was found preventable by sulfasalazine, probably via blockage of NF- κ B activation,⁸⁷ as well as the cyclooxygenase-2 inhibitor meloxicam⁹⁷ and the 12/15-lipoxy-

genase inhibitors baicalein and cinnamyl-3,4-dihydroxy- α -cyanocinnamate.⁶²

SPONTANEOUS INFLAMMATORY DIABETIC NEUROPATHIC PAIN

The mechanical and thermal behavioral tests described above provide acute sensory stimuli and represent measures of the threshold to nociceptive pain that is transduced by myelinated and unmyelinated fibers, respectively. The formalin test principally differs from both as the second phase of the flinching response occurs despite minimal input to the spinal cord from primary afferent nociceptors.³ Thus, the test can be used for studying spontaneous inflammatory nociceptive behavior in pathological conditions, including diabetes. In animal models, whereas STZ-diabetic rats display exaggerated flinching behavior in both the first and second phases of the formalin test,¹² STZ-diabetic mice lack the second phase⁹⁸ and are, therefore, unsuitable for the studies of spontaneous tonic pain associated with diabetes.

Several pathogenetic mechanisms have been implicated in formalin-induced hyperalgesia in STZ-diabetic rats. Those include increased activities of AR,⁹⁹ PARP,^{12,44} and cyclooxygenase-2⁹⁹; impaired neurotrophic support¹⁰⁰; and increase in the inhibitory neurotransmitter GABA and depletion of spinal potassium-chloride cotransporters.¹⁰¹ Correspondingly, diabetes-associated formalin-induced hyperalgesia was suppressed by several AR^{99,102} and PARP^{12,44} inhibitors, prosaposin-derived peptide,¹⁰⁰ and the GABA_A receptor antagonist bicuculline¹⁰¹ as well as insulin,¹⁰³ gabapentin,¹⁰⁴ and a B₁, B₆, and B₁₂ vitamin cocktail.⁹⁶

SUMMARY OF THE CURRENT KNOWLEDGE ON THE KEY MECHANISMS OF DIABETIC PAINFUL AND INSENSATE NEUROPATHY AND CLINICAL STUDIES OF PATHOGENETIC AND NEW SYMPTOMATIC TREATMENTS

An analysis of the literature on neuropathic sensory disorders and pain in diabetic animal models unravels key pathogenetic mechanisms implicated in several types of hyperalgesia and abnormal sensation. Those include increased AR activity, oxidative-nitrosative stress, protein kinase C, PARP and ACE activations, C-peptide deficiency, impaired neurotrophism, and proinflammatory response (probably mediated by cyclooxygenase-2, 12/15-lipoxygenase, and NF- κ B activations).

The importance of increased AR activity has been confirmed in several clinical trials of AR inhibitors. In particular, a 52-week multicenter (78 centers in Japan) placebo-controlled double-blind parallel group study of the AR inhibitor fidarestat in 279 patients with type 1 and

type 2 diabetes and associated peripheral neuropathy revealed beneficial effects of treatment on subjective symptoms including numbness, spontaneous pain, sensation of rigidity, paresthesia in the sole upon walking, heaviness in the foot, and hypesthesia.¹⁰⁵ Similar results have been achieved in the 3-year, multicenter (112 centers in Japan), comparative Aldose Reductase Inhibitor-Diabetes Complications Trial in 594 subjects with diabetic neuropathy, in which numbness of limbs, sensory abnormalities, and cramping were alleviated in patients treated with the AR inhibitor epalrestat compared with the placebo group.¹⁰⁶ A more recent open-label pilot study (12 hospitals in Japan, 22 patients) revealed beneficial effect of fidarestat on vibration perception threshold of the upper and lower limbs and subjective symptoms of PDN, such as severity of numbness in the lower limbs, heaviness in the foot, coldness and hot flushes in the lower limbs, smarting pain causing difficulty in walking, sensation as if walking on sand, sensation as if walking on an uneven road, spontaneous pain in the lower limbs, and dizziness.¹⁰⁷ The clinical trial of another AR inhibitor, ranirestat, revealed improvement of vibration perception threshold in subjects with diabetic neuropathy.¹⁰⁸ Beneficial effects on signs and symptoms of abnormal sensation and neuropathic pain have been reported in clinical studies with other agents affecting pathogenetic mechanisms of disease.¹⁰⁹ In particular, a 6-month, randomized, double-masked, placebo-controlled study of the PKC- β inhibitor ruboxistaurin revealed beneficial effect of treatment on Neuropathy Total Symptom Score-6, which quantifies frequency and intensity of aching, burning, prickling and lancinating pain, numbness, and allodynia in patients' feet and legs as well as symptom subscore and total score of Norfolk Quality-of-Life Questionnaire for Diabetic Neuropathy (composed of questions related to patients' signs and symptoms, as well as to the impact of PDN on patients' activities of daily life).¹¹⁰ The SYDNEY trial of α -lipoic acid, 600 mg d⁻¹, in 120 subjects with symptomatic diabetic sensorimotor polyneuropathy demonstrated significant improvement of the Total Symptom Score (TSS), a measure of positive neuropathic sensory symptoms, as well as of each TSS item (lancinating and burning pain, asleep numbness and prickling).¹¹¹ The SYDNEY 2 trial in 181 subjects with PDN revealed efficacy of three doses of α -lipoic acid (600, 1200, and 1800 mg d⁻¹) in improving the TSS, stabbing and burning pain, the Neuropathy Symptoms and Change Score, and the patients' global assessment of efficacy of treatment.¹¹² A double-blind, placebo-controlled, randomized multicenter study in 161 type 1 diabetes patients with diabetic distal symmetric polyneuropathy revealed beneficial effect of C-peptide on vibration perception thresholds.¹¹³ Another study revealed beneficial effect of the ACE inhibitor lisinopril on temperature discrimination and vibration perception thresholds in diabetic subjects with hypertension.¹¹⁴

Several drug candidates for the treatment of diabetic neuropathic pain are emerging from recent clinical studies of nonpathogenetic therapies. Two randomized, double-blind, placebo-controlled studies of lamotrigine, an anticonvulsant drug used in the treatment of epilepsy, in patients with painful diabetic neuropathy (each study with $n = 360$) revealed greater reduction in pain-intensity score in patients receiving lamotrigine 400 mg, compared with the placebo-treated group.¹¹⁵ Another anticonvulsant drug, topiramate, was found effective in promoting pain relief in subjects with moderately to severe painful neuropathy.¹¹⁶ Lacosamide, 400 mg, an enhancer of slow inactivation of voltage gated sodium channels, caused a statistically significant (over placebo) improvement in Likert pain score in the multicenter, randomized, placebo-controlled, double-blind trial of two doses of this agent in subjects with painful neuropathy.¹¹⁷ A randomized, blinded trial of intramuscular gene transfer using plasmid vascular endothelial growth factor to treat diabetic polyneuropathy ($n = 50$) reveal efficacy against both sensory loss and diabetic neuropathic pain.¹¹⁸

STRATEGIES FOR DRUG DISCOVERY

A recent characterization of manifestations of small sensory fiber neuropathy in diabetic rat and mouse models facilitates selection of appropriate animal models for studying diabetic painful and insensate neuropathy. Early diabetic rat models such as STZ-diabetic and Zucker diabetic fatty rats are suitable for studies of thermal hyperalgesia and pain on pressure characteristic for early PDN, and the STZ-diabetic model is also appropriate for studies of tactile allodynia and spontaneous pain (formalin-induced hyperalgesia). Rats with longer duration of STZ-diabetes, as well as STZ-diabetic *ob/ob* mice and high-fat diet-fed mice, represent good models for studying thermal and mechanical hypoalgesia characteristic of advanced PDN in humans (insensate neuropathy). Discovery of new drug targets for treatment of diabetic neuropathic pain and sensory loss should be conducted using appropriate animal models.

An analysis of the literature on experimental peripheral diabetic neuropathy suggests that, to date, all of the pharmacological agents shown to counteract one or several manifestations of painful or insensate neuropathy also have efficacy against nerve conduction velocity deficit. Note, however, that the number of experimental diabetic neuropathy-related studies with motor and sensory nerve conduction velocities as endpoints greatly exceeds the number of those in which nerve conduction measurements were combined with assessment of functional indices of small sensory fiber neuropathy. There is, therefore, a high probability that the agents previously demonstrated to prevent or reverse nerve conduction def-

icit associated with experimental PDN will also be effective against diabetes-related sensory disorders.

Diabetic neuropathic pain and sensory loss represent manifestations of chronic complications of diabetes or end-organ disease that affects the cardiovascular system, ocular lens and retina, renal cortex, and somatic and autonomic nervous systems. Studies in diabetic animal and cell culture models revealed that many pathogenetic mechanisms implicated in diabetic endothelial and myocardial dysfunction, neuropathy, nephropathy, retinopathy, and cataract are similar. In particular, numerous reports suggest the importance of increased AR, PKC, and PARP activities, nonenzymatic glycation/glycoxidation, and oxidative stress in the development of diabetic cardiovascular disease, ocular complications, and nephropathy, as well as diabetic painful and/or insensate neuropathy. It is therefore reasonable to test any new prospective pathogenetic treatments emerging from experimental studies related to other diabetic complications on indices of sensation and pain.

Valuable information regarding potential mechanisms of diabetic neuropathic pain and abnormal sensory responses, as well as new drug targets, can be obtained from studies in other (nondiabetic) models of neuropathic pain, provided that these models develop pathobiochemical changes similar to those in diabetes. For example, glutamate excitotoxicity has been implicated both in diabetic neuropathic pain^{33,119} and in neuropathic pain caused by other mechanisms,^{120,121} and STZ-diabetic rats accumulate glutamate in their peripheral nerves.^{35,36} Glutamate concentrations in the peripheral nerve system are reduced by AR inhibitor,³⁵ vasodilator,¹²² and antioxidant¹²³ treatments—and all these treatments also alleviate symptoms of diabetic neuropathic pain in diabetic animal models^{19,20–22,77,78,99,102} and in human subjects.^{105–107,111,112,114,124} Several reports indicate that the pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) plays a role in nondiabetic painful neuropathy.^{125–127} It is quite plausible that it also plays a role in diabetic neuropathic pain (as has been suggested^{128,129}), because TNF- α expression is increased in tissues of diabetic animals, and TNF- α induces overexpression of cyclooxygenase-2,^{130,131} also implicated in diabetic hyperalgesia and allodynia.^{97,99} TNF- α antagonists have never been tested in models of diabetic painful neuropathy. Other mechanisms contributing to neuropathic pain include activation of MAPKs (p38-MAPK) in spinal microglia,¹³² Schwann cells,¹³³ and macrophages¹³³ and altered calcium homeostasis and signaling in neurons.¹³⁴ These pathobiochemical changes have been documented in diabetes,^{29,132} which provides a rationale for evaluation of p38 MAPK inhibitors and calcium antagonists in diabetic painful neuropathy.

In conclusion, despite limitations of existing animal

models for studying diabetic neuropathic pain and sensory loss, evaluation of experimental behavioral correlates of clinical endpoints in diabetic animal models has dissected a number of pathogenetic mechanisms implicated in diabetic painful and insensate neuropathy. The important roles for increased aldose reductase and protein kinase C activities and enhanced oxidative stress originally revealed in experimental studies have been confirmed in clinical trials in subjects with diabetic sensorimotor polyneuropathy. New drug candidates for potential pathogenetic treatments of diabetic neuropathic pain and sensory loss can be identified through specific studies in adequate animal models, as well as analysis of the literature related to diabetic neuropathy, other diabetic complications, and peripheral neuropathic pain in conditions other than diabetes.

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