

Rodent Models of Painful Diabetic Neuropathy: What Can We Learn from Them?

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Abstract

Diabetic peripheral neuropathy (DPN) is the most common clinical complication of diabetes mellitus, and can be related to type 1 as well as type 2. To date, this highly invalidating neurological impairment is insufficiently known, understood and the treatments proposed by physicians are still empirical and poorly efficient.

Animal rodent modeling of clinical DPN offers a powerful tool in order to understand diabetes-mediated peripheral nerve injury. The majority of studies which have investigated DPN in rodent used the streptozotocin-induced rat model which reproduces metabolic lesional mechanisms of Type 1 Diabetes Mellitus (T1DM) and usual symptoms of evoked pain. Although the clinical relevance of this model is challenged due to 1) a high prevalence of type 2-compared to type 1-diabetes in the adult population, 2) the important alteration of the general clinical state of the animals and 3) the lack of morphological changes in peripheral nerves, many studies have contributed to a better pathophysiological and pharmacological understanding of the DPN.

In this review we investigated rodent models of T1DM and T2DM, their contributions for a better understanding of DPN, molecular targets and pharmacological strategies, which could be used for the enhancement of clinical care. Finally, we proposed possible ways to improve animal modeling.

Introduction

Neuropathic pain has recently been redefined by the Neuropathic Pain Special Interest Group (NeuPSIG) to correspond to "pain initiated or caused by a primary lesion or dysfunction in the nervous system" [1]. Its prevalence in general population, all causes combined, is estimated at 1.5 [2] and 6.9% [3]. The development of experimental models of neuropathic pain secondary to lesions of traumatic origin (constriction, partial section, infraorbital nerve ligation, spinal nerve ligation [4-6], etc), metabolic (diabetes, [7]) or toxic (anticancer agent [8] or retroviral [9]) has contributed to a better understanding of their pathophysiology. These models try to stand as close as possible to the symptomatology and / or clinical etiopathogeny of neuropathic pain and are currently used to evaluate new therapeutic drugs.

The need for modeling diabetic neuropathic pain comes from a clinical reality: diabetes is one of the largest providers of neuropathy in the world. Indeed, of 246 million diabetic patients, between 20 and 30 million are affected by symptomatic diabetic neuropathy [10]. Neuropathy occurs for both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), suggesting that hyperglycemia is the primary etiologic factor [11]. The most frequent clinical form is by far the diabetic distal sensory or sensorimotor polyneuropathy, affecting 30% of community-based people with diabetes [12]. Sensory polyneuropathy presents a typical distribution "in stocking and glove", and can sometimes be asymptomatic but usually causes abnormal sensations (paresthesia and dysesthesia) and/or pain. Here, the longest fibers are first affected, which explains the distal distribution. Continuous or intermittent, spontaneous or evoked, pain and abnormal sensations precede or accompany the neuropathy. Estimated prevalence of painful polyneuropathy varies between 8 and 65% [13-16] according to studies and the diagnostic tools used. Indeed, using the DN4 pain questionnaire, an overall prevalence of painful diabetic peripheral neuropathy of 14% [17] or 65.3% [14] was found. By using the Michigan Neuropathy

Screening Instrument (MNSI) and questions from the Brief Pain Inventory (BPI), the prevalence rate of painful diabetic peripheral neuropathy was 8% (MNSI score of 7 or higher and a 24 h average pain rating BPI greater than 0) [16]. Just like neuropathic pain of other etiology, diabetic neuropathic pain responds poorly to classical analgesics (acetaminophen, NSAIDs) and the reference treatments are only partially effective. Three molecules have specific authorization in this indication: gabapentin and pregabalin, calcium channels $\alpha_2\delta$ subunit ligands, and duloxetine, a serotonin and norepinephrine reuptake inhibitor (SNRI).

From these clinical settings, experimental rodent models of spontaneous diabetes were developed (*Type 1 diabetic insulinopenic BB / Worcester Rats*, *type 2 diabetic hyperinsulinemic BBZDR / Worcester Rats*, *NOD Mice*, *LETL Rats*, *Akita Mice spontaneous type 1 diabetes [B6Ins2(Akita)]*) or obtained by dietary manipulations (overeating, fasting, shift from a high fat diet to a high carbohydrate diet) (*High-fat diet-fed Mice*), genetic manipulations (*Zucker diabetic fatty rat*, *Obese leptin-deficient (ob / ob) Mice*, *Leptin receptor deficient (db / db) Mice*, *nonobese diabetic Mice*) or chemo-induced pancreatic toxicity (streptozotocin (STZ), alloxan (ALX)) [18,19].

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Experimental Models of T1DM Induced by Chemical Pancreatectomy in Rats

Two agents can be used to induce chemical pancreatectomy, both are glucose analogs: ALX, a pyrimidine derivative (synthesized in 1938) and STZ, an alkylating and antimicrobial agent. Chemical properties of these compounds are crucial for their ability to induce diabetes [20]. Both are hydrophilic and cannot cross plasma membrane. They use the glucose transporter GLUT2, which is expressed by the pancreatic beta-cells.

Cytotoxic effects of ALX are due to its reduced reaction product, dialuric acid, and to the production of reactive oxygen species (ROS) (superoxide radicals $O_2^{\cdot-}$, hydrogen peroxide H_2O_2 and hydroxyl OH^{\cdot} , [21]).

STZ exerts its toxicity through DNA alkylation [21]. Protein glycosylation is an additional deleterious factor. STZ induces ADP polymerase over-stimulation leading to a decrease in NAD^+ as well as in ATP concentration and leads to the activation of apoptotic program that destroys beta-cells and all the cells expressing the GLUT2 transporter (cells from the kidney and liver). By performing a bibliography research using the database MEDLINE (PUBMED) and the following keywords: "diabetes" and "alloxan" or "streptozocin" and "neuropathy" during the last 30 years (i.e. 1982 to 2012), 298 studies used the antimicrobial agent STZ and only 48 used ALX to induce diabetes. During the last 10 years (i.e. 2002 to 2012) the ratio ALX: STZ was 9: 139 probably due to the poor specificity of alloxan compared to STZ against pancreatic beta-cells. Indeed STZ generally produces greater cytotoxicity due to its conversion to anionic radicals.

STZ is more commonly used because of its greater stability and relative lack of extrapancreatic toxicity [22]. Thus, we focused our review on STZ-induced diabetic neuropathy in rodents.

Clinical signs of STZ-induced diabetes in rats

After STZ administration, hyperglycemia and hypoinsulinemia appear in the first days and persist, attesting to an irreversible toxicity. A halt in weight growth and sometimes even a weight loss are also observed [7]. Hyperglycemia is concomitant with polydipsia (water intake 10-times higher), polyuria and polyphagia [23]. While most morphological, histological and electrophysiological studies show that diabetic neuropathy is accompanied by nerve structural changes (segmental demyelination and axonal degeneration) and functional changes (assessed by nerve conduction velocity) in diabetic patients [10], structural changes are rarely reported in STZ-induced diabetic rats or appear slowly and later. Walker et al. [24] using tibial nerve biopsies from diabetic rats reported the lack of abnormal nerve tissue regarding the distribution of unmyelinated axons, diameter of myelinated axons, fascicular area, absence of Wallerian degeneration. However, abnormalities in the structure of endoneural capillaries presented increased luminal surface and decreased endothelial cells size, related to impairment in *vaso nervorum*. Therefore, in this experimental model, there is no structural support for the functional abnormalities, and changes in pain sensitivity.

In the absence of segmental demyelination and axonal degeneration that characterizes human diabetic neuropathy, the diabetic rat model of STZ could be considered as a short duration model of hyperglycemia in which functional abnormalities reflect early stages of diabetic neuropathy.

Etiopathogenic factors of STZ-induced neuropathy

The involvement of chronic hyperglycemia in the development and aggravation of T1DM complications in humans has been confirmed by a North American multicentric study (Diabetes Control and Complications Trial Research Group, 1993) performed on 1441 patients followed during 6.5 years. In this study, the prophylactic importance of glycemic control on the progression of retinopathy (34-76% reduction), microalbuminuria (50%) and neuropathy (60% reduction) was further supported. Etiopathogenic factors of sensory neuropathy are still unclear but hypothesis coming from experimental work on diabetic rats were made. Several mechanisms underlying glucotoxicity on peripheral nerve fibers have been proposed [25]: an enzymatic mechanism involving the polyol pathway, proteins glycation and expression of advanced glycation end products receptors, as well as oxidative stress.

Glucose uptake by Schwann cells of nerves is independent of insulin: glucose enters and accumulates in neurons initiating the aldose reductase pathway. This metabolic pathway leads to the accumulation of sorbitol and fructose, to the depletion of myo-inositol and compromises glutathione cycle and ATPase Na^+/K^+ activity. Inhibitors of aldose reductase, that are very efficient on functional impairment due to diabetes in rats, are much less efficient in diabetic patients, a difference probably related to the importance of the polyol pathway in rodents compared to humans.

Hyperglycemia also induces a non-enzymatic glycation of proteins, glycation end products in turn activate the transcription factor NF κ B responsible for the modification of many genes expression.

Finally, excess of glucose in neuron is responsible for the increase of oxidative stress by combining free radical genesis and inefficient antioxidant protection systems. Most ROS ($O_2^{\cdot-}$, OH^{\cdot} , H_2O_2) are produced by the mitochondrial respiratory chain; NADPH oxidase and xanthine reductase, as well as reactive species of nitrogen (nitric oxide NO, peroxynitrite, ONOO-) produced by the NO synthesis enzyme NO-synthase, have been shown to be involved in the development of diabetic peripheral neuropathy in STZ-treated rat. In STZ rats, it was shown that free radicals exerted their deleterious effects on Schwann cells. Chain reaction neutralization generated by ROS is assessed by superoxydismutase (SOD), catalase and glutathione peroxidase. Thus treatment of STZ rats with antioxidants not only prevents or suppresses functional impairment [26], but also the pain-related behaviors [27]. Conversely, treatment of healthy rats with a pro-oxidant agent (premaquine) induces functional changes similar to those observed after induction of diabetes [28].

Some signaling pathways involving the MAPKinases are activated in sensory neurons exposed to increased glucose *in vitro* and *in vivo* in rats and humans with diabetes [29].

Hypersensitivity in STZ-induced diabetic rat

Behavioral studies assessed in STZ-diabetic rats often focus on their response to nociceptive or non-nociceptive stimuli, because of the absence of quantifiable signs of spontaneous pain. These tests consist in measuring time latency or withdrawal thresholds of an animal whose paw or tail is exposed to a thermal, mechanical or tactile stimulation. The place preference test, where the animal can chose between two temperatures [30] presents the advantage of getting rid of animal handling, therefore allowing the assessment of spontaneous behavior towards a range of thermal stimuli, leaving the animal free to stand on one of the

two plates of different temperatures. Using this test has allowed to reveal a thermal hypersensitivity (for a temperature of 45°C) in STZ rats [31]. However, thermal hyperalgesia towards hot temperatures is not a common painful symptom in diabetic patients, which makes difficult the extrapolation of these results toward clinic [32]. Some authors, using the thermal ramp test that consists in placing the animal on a surface which temperature increases of 1°C/sec from 30°C to 50°C, have also observed hypersensitivity during the first few weeks of diabetes but that transforms into a hypoalgesia 2 to 3 month later, signing an evolution of the painful neuropathy toward an insensitive neuropathy towards nociceptive hot stimuli, that can be found in humans [33]. Moreover, a loss in thermal nociceptors was reported in diabetic patients [34].

The perception of tactile stimuli (light touch) and mechanical (pressure) is also affected by diabetes. In STZ rats, the application of a von Frey filament, producing a light static touch, causes a paw withdrawal induced by the inappropriate activation of A δ and C fibers, signing a tactile allodynia. More recently, cotton swabs or brushes have been used to measure dynamic tactile allodynia by caressing the plantar surface of the hind paw of the animal, which evokes a paw withdrawal if A β fibers are impaired. The comparison of the two different symptoms reveals that dynamic allodynia has a later onset than static allodynia and both painful symptoms worsen over time in STZ rats [35]. This observation suggests that the presence of dynamic allodynia results in more severe nerve damage than when static tactile allodynia is only symptom. The STZ-induced diabetic rat model makes it possible to test the dynamic tactile allodynia, which, unlike the static allodynia and thermal hypersensitivity to hot temperatures, is a common symptom of neuropathic pain in humans. The use of von Frey filaments can also be exploited for the exploration of mechanical hypersensitivity: the application of a von Frey filament (# 4.93) exerting a very static pressure point, results in a two fold increase of the intensity of the response in diabetic animals [36].

The search for a chemical sensitivity in diabetic rats, that could, at best, mimic human inflammatory hypersensitivity observed in clinic, revealed an increase in the tonic response while the phasic response is not altered by the chemical agent [37].

Pathophysiology of STZ-induced neuropathic pain

Pain associated with nerve damage from diabetes initially involves peripheral mechanisms causing sensory fibers hypersensitivity, which secondarily leads to central rearrangements responsible for central nociceptive system hyperexcitability. In this section, we discuss the main peripheral and central mechanisms of diabetic neuropathic pain proposed by the work using the STZ-rat model.

Peripheral changes: Involvement of voltage-dependent calcium channels Cav: The T-type Cav channels ("LVA" low voltage activated) or Ca_v 3.1, 3.2 and 3.3 are localized in cell bodies and dendrites of primary afferent fibers, and play an important role in modulating the neuronal excitability [38]. Their involvement in the pathophysiology of neuropathic pain has also been demonstrated, particularly in models of diabetes and traumatic neuropathies by sciatic nerve ligation, where current density of type T is greatly increased [36]. The "knockdown" strategy by Ca_v3.2 isoform antisense but not the Ca_v3.1 or Ca_v3.3 isoforms, suppresses thermal (Hargreaves test) and mechanical hypersensitivity (applying a von Frey filament # 4.93) in STZ-diabetic rats. Electrophysiological recording from small cells (C fibers) of dorsal root ganglia (DRG) and spinal cord (whole cell voltage-clamp) shows that the same strategy inhibits the "up-regulation" of T-type currents induced by diabetes [39].

Finally, over expression of the $\alpha 2\delta$ subunit of L-type calcium channels belonging to the family of "HVA" (high activation threshold) in the DRG of diabetic rats is contemporary with the development of tactile allodynia appreciated by the test of von Frey filaments [40]. This $\alpha 2\delta$ subunit is also the pharmacological target of certain antiepileptic drugs such as gabapentin and pregabalin.

Involvement of voltage-dependent sodium channels Na_v: Peripheral nerve injury can alter the expression and function of NaV channels α subunits which results in a change in neuronal excitability [41]. Hong et al. [42] have shown that four weeks after induction of diabetes by STZ in rats, the Na_v currents sensitive (S) and resistant (R) to tetrodotoxin (TTX) increased in small diameter DRG. Quantification by Western blotting of different types of sodium channels showed an increased expression of Na_v1.3 and NaV1.7 (TTX-S) channels and a decreased expression of NaV1.6 (TTX-S) and NaV1.8 (TTX-R) channels in DRG of diabetic rats (four weeks post-STZ). These authors also reported that phosphorylation of Thr / Ser residues of Na_v1.8 and Na_v1.6 channels, and Tyr residues of Na_v1.7 and NaV1.3 channels is increased by diabetes. This fact is not unambiguous: while an increase and a decrease in Na_v1.3 and Na_v1.8 channels expression (mRNA and protein) respectively, have already been found, the expression of Na_v1.6 (mRNA and protein) has been shown to increase [43]. Sensitive or resistant TTX Na_v channels play an important role in the pathophysiology of neuropathic pain of all etiologies, including diabetic, by changing the electrical properties of the membrane, thus contributing to the genesis of ectopic discharges. These channels are also the target of different molecules (tricyclic antidepressants, anticonvulsants, local anesthetics ...) which therapeutic efficacy in the treatment of neuropathic pain is established.

Involvement of Transient Receptor Potential (TRP) channels: Thermal sensitivity observed in STZ-treated animals [31] is probably due to the sensitization of cutaneous nociceptors associated with A δ and C fibers. TRPV1 channel (Transient Receptor Potential Vanilloid type 1), is a major actor in thermal sensitivity, predominantly present in C fibers and, to a less extent, in A δ fibers [44-46]. TRPV1 is a non-selective calcium/sodium-permeable channel activated by temperatures up to 43°C, capsaicin (extracted from red pepper), protons (pH < 5.9), metabolites of arachidonic acid ... ; TRPV1 can be sensitized by phosphorylation, by prostaglandins, bradykinin, glutamate, histamine, serotonin, ATP or NGF. Any change in TRPV1 expression, associated with changes in intracellular signal transduction, may lead to spontaneous neuronal activity induced by normal body temperature; this is the case if the response threshold of TRPV1 is lowered below 38°C [47]. Pabidi et al. [45], reported an increase in the amplitude of TRPV1 currents induced by capsaicin in STZ-induced hyperalgesic mice compared to STZ-induced hypoalgesic mice or normoglycemic control mice. The expression of TRPV1 channels in A δ and C fibers of STZ-treated mice was increased in those presenting hyperalgesia, and reduced in hypoalgesic mice. The same team also showed that thermal hypersensitivity developed by diabetic *wild-type* mice is abolished when the gene coding for TRPV1 channel is disabled (TRPV1^{-/-} mouse). Finally, treatment with anti-vanilloid VR1 receptor antiserum abolishes thermal hyperalgesia in STZ-treated mice [44]. In physiological conditions, it was shown that insulin positively modulates the activity and expression of TRPV1 channels via protein kinase C (PKC) [48]. It is therefore possible that the sudden decrease in insulin levels induced by STZ is indirectly responsible for a decreased in TRPV1 activity, which would lead to a compensatory increasing of the expression of these channels. This could explain the thermal hypersensitivity appearing in the third

week of diabetes in our study [31]. Another hypothesis brought by Pab-bidi et al. [48] suggests a direct action of STZ onto sensory neurons, involving the ROS-p38 MAPkinase pathway, thereby altering expres-sion and function of TRPV1. However, a direct effect of STZ on the expression and / or functionality of TRPV1 can be excluded because we have shown that animals which failed to develop hyperglycemia after STZ injection did not present thermal sensitivity disorders (unpub-lished results).

On the other hand, a second TRP channel, TRPA1 (Transient Re-ceptor Potential Ankyrin type 1) seems to be involved in DPN, since some studies showed that TRPA1 antagonists changed mechanical thresholds in STZ-treated rat [49,50]. Moreover, the TRPA1 channel can be activated in sensory neurons by ROS, alkenyl aldehydes and 15-deoxy-prostaglandin J_2 , which are generated during oxidative stress leading to intracellular calcium rise [51,52]. Hence, TRPA1 receptor through indirect activation by metabolites from oxidative stress seems to be an important molecular protagonist in mechanical hypersensitiv-ity of DPN.

Involvement of HCN channels: Described for the first time in pacemaker cells of the heart sinus node [53], HCN (hyperpolarization-activated cyclic nucleotide-gated cation) channels were discovered in neurons and responsible for Ih currents [53]. HCN channels open when the membrane is hyperpolarized (-60 to -50 mV, i.e. to the rest potential) and generate a mixed Na^+ , K^+ cationic current. Four genes coding for HCN channels have been identified (HCN1-4). The most abundant in neurons of DRG are the HCN1/2 type. The Ih current gen-erated is of greater amplitude, faster and more frequent in neurons of large and medium diameter (type A) than in small diameter neurons (type C). The administration of a HCN channel blocker, the ZD7288, suppresses tactile allodynia in STZ-induced diabetic rats (three weeks post-STZ) and reduces mechanical hypersensitivity as well (personal results), whereas ivabradine, a more selective blocker of HCN channels, suppresses cold allodynia in a model of toxic neuropathy induced by oxaliplatin [54].

Together, these data obtained in diabetic rats underline the impor-tant role played by ion channels in the balance of the neuronal mem-brane and the importance of any change in expression levels or thresh-olds of activation of these channels on the excitability of sensory fibers and their deleterious effects on nociception.

Central changes

Involvement of N-Methyl-D aspartate (NMDA) receptors: *In vivo* analgesic effects of dizocilpine, memantine or D-CPP, NMDA receptor non-competitive and competitive antagonists respectively, on mechani-cal hypersensitivity in STZ rats [55-57] can also be obtained in human clinical studies with ketamine, but they unfortunately induce debilitat-ing side effects [58], which compromise their clinical use. NMDA re-ceptor phosphorylation would be involved in the development of tactile allodynia, mechanical and thermal hypersensitivity [23]. Our team has also shown the importance of the specific activation of certain isoforms of MAPKinases in painful hypersensitivity in STZ animals, as well as the need for NMDA receptor activation for the phosphorylation of these kinases [59], opening new prospects for a more targeted drug therapy, thus better tolerated for diabetic neuropathic pain.

Alteration of descending systems: One of the pathophysiological mechanisms involved in the pathogenesis of chronic pain including neuropathic pain, is a loss of the inhibitory role of serotonin on persis-tent pain, as evidenced by (i) the nearly ineffectiveness of selective sero-tonin reuptake inhibitors (SSRIs) in neuropathic pain patients [60] and,

(ii) results obtained in STZ-induced diabetic rats showing an alteration of spinal 5-HT_{2A} receptor-mediated analgesic effect, usually involved in the analgesic effect of serotonin [31]. These receptors have the particu-larity to be associated with specific multiprotein complexes, consisting in part of proteins containing PDZ domains, which can modulate signal transduction of receptors to which they are associated [61]. In STZ rats, the administration of a cell-penetrating peptidyl mimetic of the 5-HT_{2A} receptor C-terminus ending, which disrupts their interaction with PDZ proteins, induces antihyperalgesic effect *per se* and enhanced the anal-gesic effect of fluoxetine, an SSRI [31].

Most of peripheral and central abnormalities in the transmission and modulation of nociception that have been described in STZ rats and were also found in other neuropathic pain models, especially trau-matic peripheral nerve injury (CCI or SNL) showing the lack of speci-ficity of the model. It would be simplistic to want to associate a patho-physiological mechanism to an etiology because the same mechanism can be found in neuropathies of different etiologies [62], and a given injury may involve several mechanisms.

Activity of reference drugs

Ca_v channels $\alpha_2\delta$ subunit ligands: The antiallodynic and analge-sic efficacy of pregabalin [63] and gabapentin [55], whose action de-pends on binding to the $\alpha_2\delta$ -1 subunit of the Ca_v2.X, has been shown in many neuropathic pain models, including STZ-induced diabetic rat. The increased expression of mRNAs encoding subunits $\alpha_2\delta$ of neurons of small (C fibers) and medium (A δ) caliber in diabetic rats has been known for a decade [40] and would play a major role in the develop-ment of pain hypersensitivity.

N-type Ca_v channel (Ca_v2.2) blockers: Leconotide and ziconotide, synthetic versions of ω -conotoxins MVIIA and CVID produced by marine mollusks, showed dose-dependent analgesic activity after in-travenous administration in the diabetic neuropathic pain model , on thermal hyperalgesia [64]. However, only the intrathecal route of administration of ziconotide is effective in patients suffering from se-vere chronic pain, emphasizing the difficulties and precautions needed when extrapolating data obtained from animal experiments to human disease.

Na_v channel blockers: Na_v channels are the target of many anal-gesics. Topical lidocaine (patch form), prescribed for the treatment of postherpetic neuropathic pain, is one of the most used sodium channel modulator in human therapeutics. Its analgesic activity when systemi-cally administrated had been reported in the model of diabetic neu-ropathy [37,65]. Having a similar structure, mexiletine showed anti-al-lodynic activity during the early stages of experimental diabetes (three weeks post-STZ) [63], suggesting a reorganization of sodium channels along diabetes.

Antidepressants: Literature which report the analgesic effect of an-tidepressants in animal models are numerous and heterogeneous. We have recently stressed out the importance of using protocols of admin-istration similar to those used clinically (repeated administration every half-life time) for assessing the effects of antidepressants [66]. Using chronic treatment, we have highlighted a differential profile of activ-ity of milnacipran (a SNRI) depending in the etiology of neuropathy, and proposed selection criteria to use dual monoaminergic antidepres-sants based on their opioidergic mechanism: such mechanism would be predictive of modest efficacy, regardless of the neuropathy etiology [66]. Other experimental works in animal models, based on compara-

tive studies of the mechanism of action of antidepressants according to the etiology and symptomatology of neuropathic pain, are necessary before initiating clinical trials; those clinical trials will be useful to test whether semiologic profiles of responders or nonresponders to a particular antidepressant may be considered as selection criteria, source of personalized treatment and thus improved efficiency.

Despite the relative lack of pathophysiological specificity of the diabetic model, pharmacological data showed particular activity profiles of analgesics that are considered to be efficient in this painful context, and made possible recommendations to improve the clinical relevance of the model.

When one considers that the predictability of a model refers to its symptoms and its sensitivity to pain treatments recognized as effective in a painful context, the experimental model of diabetic neuropathy induced by STZ can be considered as predictive, bearing in mind that the spontaneous pain has never been quantified in this model, probably because of the lack of appropriate behavioral tools. Improvement of its clinical relevance is still necessary and may come from a standardization of procedures regarding the selection of the animal (strain, age, sex, breeding), the chronicity of the disorder, the pain measurement (operative and non operative testing) [67], or on how to use pharmacological tools.

Type 2 Diabetes Model in Rodents

Type 2 diabetes (T2DM) is the most representative form of diabetes mellitus in adult diabetic population. T2DM has affected 285 million people worldwide in 2010 [68], and will probably affect more than 366 million in 2030 [69]. T2DM is characterized by an impairment of insulin actions caused by insulin secretory defects and/or peripheral insulin resistance. Peripheral insulin resistance is compensated by increasing insulin secretion which leads to reduced pancreatic beta-cell (insulinopenia) functions through local inflammatory processes which drive to increase again glycaemia [70]. The prevalence of DPN is higher in type 2 (50.8%) than in type 1 (25.6%) diabetic patients; the prevalence of painful DPN is 14% which, again, is higher in type 2 (17.9%) than in type 1 (5.8%) patients [17]. Nevertheless, only few studies focused their efforts to develop correct rodent model for investigating of T2DM-induced neuropathy and develop new strategies against peripheral neuropathy. A PUBMED search with « type 1 diabetes neuropathy » finds 2349 matches which have been published between 2012 and 1964 whereas a search with « type 2 diabetes neuropathy rat » or « type 2 diabetes neuropathy mice » finds 32 and 41 matches respectively which have been published between 2012 and 1988. In this sense, we have selected for this review 29 articles, which explored pain behaviors, pain thresholds and/or nerve conduction velocity in T2DM-induced neuropathy (Table 1 and 2).

Obese models of T2DM

In rat almost 50 % of articles worked in the Zucker Diabetic Fatty model (ZDF), a useful and well-known model of leptin receptor gene deficiency, which displays hyperphagia, fat overstorage, glucose intolerance, hyperglycemia, glucosuria, and polyuria. Authors using this model reported tactile allodynia, mechanical and thermal hyperalgesia and a decrease in nerve conduction velocity [71-75]. In ZDF rats, the number of sural axons is preserved, but atrophy and a loss of large-caliber dermal and small-caliber epidermal axons are observed [71]. Otto et al. [73] recently showed a temporal loss of opioid sensitivity in these animals and a marked morphine hyposensitivity was evident at

six months. Romanovsky et al. [74] also showed that the compensation of hyperinsulinemia might not restore compromised nerve function. On the other hand, Li et al. [72] showed that a 2% taurine diet reverses mechanical hypersensitivity and neurovascular deficits. Eventually, Sugimoto et al. [75] showed that ZDF animals also exhibited progression from thermal hyperalgesia to hypoalgesia, which occurred more rapidly and coincided with a rapid decline in pancreatic insulin secretion.

The same model of obesity and T2DM is available in mice since *ob/ob* and *db/db* models, which display leptin and leptin receptor deficiency respectively, have been developed. These models are often used for the assessment of T2DM-induced neuropathy in mice. The most obvious characteristic of leptin-deficient *ob/ob* mice is that they are grossly overweight and have higher food consumption. They are also hyperglycemic, hyperlipidemic, hyperinsulinemic and display lowered physical activity [76]. *Db/db* mouse is the most widely used model for the study of T2DM neuropathy in mice. First described in 1966, the *db* gene encodes a G-to-T point mutation to the leptin receptor, which is transmitted in an autosomal recessive fashion. This defect leads to the development of hyperphagia, obesity, hyperlipidemia, hyperinsulinemia, insulin resistance, and diabetes [77].

Ob/ob mice [78-80] and *db/db* mice [81-83] develop thermal hypoalgesia, tactile allodynia and a decrease in nerve conduction velocity. *Ob/ob* mice developed manifest sciatic motor nerve conduction velocity (MNCV) and hind-limb-digital sensory nerve conduction velocity (SNCV) deficits, thermal hypoalgesia, tactile allodynia, and a remarkable loss of intraepidermal nerve fibers [80]. In this mice, administration of fidarestat, an aldose reductase inhibitor, was associated with preservation of normal MNCV and SNCV, alleviation of thermal hypoalgesia and decreasing of intraepidermal nerve fiber loss, but not tactile allodynia [78]. Sciatic nerves of *wild type* C57BL6, *ob/ob*, and *db/db* mice were investigated by electronic microscopy, which revealed injuries in myelin sheaths in small (< 5 µm), medium-sized (5-10 µm), and large axons (>10 µm) of *db/db* mice compared with *wild type* mice. In *ob/ob* mice, only large fibers showed a decrease in myelin sheath thickness. Moreover, the basement membranes of endoneural microvessels were thickened in both obese groups. The authors also explored laminin expression by western blot and showed a decrease in *db/db* group but not in *ob/ob*. Hence, changes in nerve fibers and in endoneural microvessels are present in sciatic nerve of both mouse models [79]. Gene expression changes in *db/db* mice are consistent with structural changes of axonal degeneration and interestingly *Nerves Growth Factor* (NGF), *Substance P* (SP), and *calcitonin gene-related peptide* (CGRP) are up-regulated in dorsal root ganglion (DRG) of *db/db* mice before or during the development of mechanical allodynia [84]. Interestingly, up-regulation of NGF coincided with enhanced tyrosine kinase A (TrkA) receptor phosphorylation in DRG. Further study aimed to identify the detailed mechanism of astrocyte-induced allodynia in *db/db* mice. Results showed that spinal activated astrocytes dramatically increased interleukin 1β expression which may induce the phosphorylation of NR1 subunit of NMDA on the serine residue 896 [81].

All these results show that T2DM neuropathy in obese rat and mice models could be sustained by direct injuries onto the peripheral nervous system, which involved classical molecular actors found in pain sciences. Nevertheless, these models of leptin-deficient or leptin receptors deficient obesity cannot represent a clinical reality since leptin mutation in human population still rare and, typically, people risking to develop T2DM or obesity, which could lead to T2DM have a complex

Animals	Strains	Model	Regimen	Age	Sex	Nociceptive behavioral test	Reported pain symptoms (hyperalgesia/allodynia)	Nerve Conduction	References
Rat	ZDF	Bio-Breeding Zucker Diabetic Rat (BBDZR)	Rat chow	8 months	Male	Plantar test	Thermal hyperalgesia	Decreased	Kamiya H et al. 2005
Rat	Wistar	Goto-Kakizaki	Rat chow	7 to 32 weeks	Male and female	Tail-Flick	Thermal hypoalgesia	Not done	Ueta K et al. 2005
Rat	Wistar	Goto-Kakizaki	Rat chow	6 weeks	Male	None	None	Decreased	Kitahara Y et al. 2002
Rat	Wistar	Goto-Kakizaki	Rat chow	18 months	Male	None	None	Decreased	Murakawa Y et al. 2002
Rat	Wistar	Goto-Kakizaki	Rat chow	10 weeks	Male	TailFlick	Thermal hypoalgesia	Not done	Liepinsh E et al. 2009
Rat	Long Evans	Otsuka Long-Evans Tokushima Fatty (OLETF)	30 % Sucrose in drinking water	30 weeks	Male	None	None	Decreased	Nakamura J et al. 1997
Rat	Long Evans	Otsuka Long-Evans Tokushima Fatty (OLETF)	Sucrose feed	2 months	Male	Tail Flick and Tail Pressure Test	Mechanical and thermal hypoalgesia	Decreased	Kamenov Z et al. 2006
Rat	SpragueDawley	STZ-induced type II Diabetes*	Standard rat diet supplemented with 10% sucrose, 10% lard, 2% cholesterol and 0,2% cholic acid + STZ 30 mg/kg	4 weeks after induction of diabetes	Male	Radian Test	Thermal hypoalgesia	Decreased	Xiu-ying Yang et al. 2011
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	8 weeks	Male	von Frey hairs Test and Plantar Test	Tactile allodynia	Decreased	Brussee V et al. 2008
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	7 weeks	Male	von Frey hairs Test	Tactile allodynia	Not done	Otto KJ et al. 2011
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	6 to 10 weeks	Male	Randal-Selitto	Mechanical hyperalgesia	Not done	Romanovsky D et al. 2008
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	6 weeks after diagnostic of hyperglycemia	Male	von Frey and thermal stimulation	Thermal hyperalgesia, Tactile allodynia.	Decreased	Li F et al. 2006
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	15 weeks	Male	None	None	Decreased	Shimoshige Y et al. 2000
Rat	ZDF	ZuckerDiabeticFatty	Rat chow	8 to 36 weeks	Male and female	Tail Flick, Randall-Selitto	Thermal hypoalgesia followed by apersistent thermal hyperalgesia and tardive mechanical hyperalgesia.	Not done	Sugimoto K et al. 2008

*Developp by Srinivasan K et al. (PharmacolRes. 2005)

Table 1: Rat models used in T2D-induced neuropathy.

association of inherited variations at many genetic sites and are exposed to environmental stressors [85]. In this sense, few non-obese but more pertinent models of T2DM were developed but, unfortunately, they are seldom used for the study of T2DM-induced peripheral neuropathy.

Non-obese model of T2DM

The best described rat model of non-obese diabetes which does not result of single point mutation is the congenic strain *Goto Kakizaki* (GK). GK is a moderately diabetic rat strain that was developed by Masaei Kakizaki and Yoshio Goto by repeated inbreeding of glucose-intolerant Wistar rats over several generations. In contrast to many other rodent models of non-insulin-dependent diabetes GK rat does not exhibit hyperlipidemia nor obesity [86].

Murakawa et al. [87] showed an impairment in the blood glucose tolerance tests in GK rats, a decrease of 76 % of normal MNCV, a loss of small myelinated fibers and an atrophy/loose of unmyelinated axons. On the other hand, the levels of NGF in the sciatic nerve were

significantly reduced, and concomitantly, TrkA and NGFp⁷⁵ receptor expression was decreased in DRG. These changes were accompanied by significantly reduced immunoreactivity for SP and CGRP in DRG neurons and sciatic nerve. Unfortunately, this interesting paper does not correlate painful thermal and mechanical thresholds with peripheral damages and impaired expression of molecular antagonists [87].

Most studies have highlighted the beneficial role of the GK model in pharmacology by testing new drugs. Ueta et al. [88] reported that GK rat presented thermal hypoalgesia and explored the anti-hypoalgesic effect of T-1095, an orally active inhibitor of Na⁺-glucose co-transporter (SGLT). Throughout the study, T-1095 treatment significantly decreased both blood glucose and hemoglobin A(1C) levels in the GK rat and a concomitantly reduced the thermal impairment in tail-flick test [88]. In the same manner, Kitahara et al. [89] examined the effect of long-term suppression of postprandial hyperglycemia and glycemic fluctuation in GK by nateglinide, an antidiabetic drug which stimulates the release of insulin from pancreatic beta cells. Nateglinide treatment

Animals	Strains	Model	Regimen	Age	Sex	Nociceptive behavioral test	Reported pain symptoms (hyperalgesia/allodynia)	Nerve Conduction	References
Mice	C57Bl6/J	High Fructose Diet	D12328, 10,5 kcal% fat, and D12330, 58kcal% fat with corn starch	16 weeks of diet	Male	von Frey hairs test, Tail Flick	Thermal and mechanical hypoalgesia, and tactile allodynia	Decreased	Watcho P et al. 2010
Mice	C57Bl6/J	High Fructose Diet	D12328, 10,5 kcal% fat, and D12330, 58kcal% fat with corn starch	16 weeks of diet	Female	von Frey hairs test, Tail Flick	Thermal hypoalgesia, Tactile allodynia.	Decreased	Obrosova IG et al. 2007
Mice	C57Bl6/J	High Fructose Diet	D12328, 10,5 kcal% fat, and D12330, 58kcal% fat with corn starch	16 weeks of diet	Female	von Frey hairs test, Tail Flick	Thermal hypoalgesia, Tactile allodynia.	Decreased	Stavniichuk R et al. 2010
Mice	C57/BL6	high-fat (45% kcal %fat) diet	45% kcal %fat diet D12451i	34 weeks	Male	Radian test	Thermal hypoalgesia	Decreased	Vincent AM 2009
Mice	C57BLKS/J	Leptin receptor-deficient db/db mice	Micechow	10-12 weeks	Male	Beam walk, Footprinting, Grid walk, Rotarod	None	Decreased	Muller KA et al. 2009
Mice	BKS.Cg-m ^{+/+} Leprdb/J	Leptin receptor-deficient db/db mice	Micechow	24 weeks	Male	Hind paw withdrawal time and Tail Flick	Thermal hypoalgesia	Decreased	Pande M et al. 2011
Mice	C57BLKS	Leptin receptor-deficient db/db mice	Micechow	?	Male	von Frey hairs test	Mechanical hyperalgesia	Not done	Cheng HT et al. 2010
Mice	C57BLKS	Leptin receptor-deficient db/db mice	Micechow	10 weeks	?	von Frey hairs test	Mechanical allodynia	Not done	Liao YH et al. 2010
Mice	BKS.Cg-m ^{+/+} Leprdb/J	Leptin receptor-deficient db/db mice	Micechow	9 to 16 weeks	Male	von Frey hairs test, Dynamic plantar aesthesiometer, Plantar test, Formalin	Mechanical hyperalgesia, hypoesthesia in second phase of formalin test	Not done	Wright DE et al. 2007
Mice	C57BLKS/J	Leptin receptor-deficient db/db mice	Micechow	1 to 5 month	Male	von Frey hairs test	Mechanical hypersensitivity	Not done	Ren YS et al. 2012
Mice	C57Bl6/J	Leptin-deficient ob/ob mice	Micechow	8 weeks	?	von Frey hairs test, Radian test, Hot plate test (55°C)	Thermal hypoalgesia, Tactile allodynia.	Decreased	Vareniuk I et al. 2007
Mice	C57Bl6/J	Leptin-deficient ob/ob mice	Micechow	6 weeks	?	von Frey hairs test, Radian test	Thermal hypoalgesia, Tactile allodynia.	Decreased	Drel VR et al. 2006
Mice	C57BL6	Leptin-deficient ob/ob mice and Leptin receptor-deficient db/db mice	Micechow	4 months	Male and female	None	None	Not done	Nowicki M et al. 2012
Mice	CD1	Stress-induced type 2 Diabetes	Micechow	30 days	Male	TailFlick	Thermal hypoalgesia	Not done	Loizzo S et al. 2010
Mice	ddY-SPF	Tsumura, Suzuki, Obese Diabetes (TSOD)	Micechow	6 and 12 months	Male	Tail pressure test (Analgesymeter)	Mechanical hyperalgesia	Not done	Iizuka S et al. 2005

Table 2: Mice models used in T2D-induced neuropathy.

suppressed postprandial hyperglycemia by 50% and normalized delayed motor nerve conduction but once again, authors do not correlate these results with pain thresholds evaluation [89]. To finish, Liepinsh et al. [90] showed that mildronate, an anti-ischaemic drug, significantly decreased both the fed- and fasted-state blood glucose and the thermal hyposensitivity [90].

The GK model is the one for which pharmacological studies have been done to study T2DM-mediated peripheral neuropathy. However it appears clearly that antidepressants, anticonvulsants, as well as $\alpha 2\delta$ ligands, which display clinical efficiency, should be investigated in this model in order to validate its clinical pertinence for the development of

new analgesic compounds.

The last model used for studying T2DM induced-neuropathy is the diet-induced diabetes model. Very few article explored pain sensitivity in this model, which, nevertheless, displays neuropathic changes when animals are fed with high fat diet (HFD). This model of T2DM-induced neuropathy is exclusively caused by the dietary regimen, the most important factor associated with idiopathic neuropathy in non-diabetic human subjects [91]. In mice, two studies explored pain thresholds in HFD which led to the conclusion that the development of thermal hypoalgesia was identical in both females [92,93] and males [94]. In females, tactile allodynia was also reported, but mechanical hypoalgesia

was only reported in males [92,93,95]. These studies showed the role of nitrosative stress in peripheral nerves and demonstrated the role 4-hydroxynonenal adduct, nitrotyrosine, poly (ADP-ribose) accumulation and 12/15-lipoxygenase overexpression in peripheral nerve and dorsal root ganglion neurons. Authors proposed that oxidative stress is a good target for the treatment of diabetic peripheral neuropathy.

Other models

T2DM-induced neuropathy was also studied in another models but their using still is marginal.

1) The *Otsuka Long Evans Tokushima Fatty* (OLETF) rat is a Cholecystokinin 1 receptor (CCK1) knockout model which allows studying the multiple CCK functions. OLETF rats are grossly hyperphagic probably due to the loss of a feedback satiety signal in the central nervous system [96]. Administration of sucrose to OLETF rats caused significant body weight increase and marked hyperglycemia. Sucrose-fed OLETF rats demonstrated significantly delayed MNCV and their thermal nociceptive thresholds is significantly decreased [97].

2) The inbred *Bio-Breeding Zucker diabetic rat* (BBZDR)/Wor, is a relatively emerging model of T2DM. Diabetic male BBZDR/Wor rat are homozygous for a leptin receptor gene mutation and shares genetic background of original BB strain. BBZDR/Wor rats are hyperlipidemic and hyperleptinemic, become insulin resistant, and ultimately develop hyperglycemia as well as thermal hyperalgesia [98].

3) *Tsumura Suzuki Obese Diabetes* (TSOD) mice, were also obtained by selective breeding of obese male mice of the ddY strain and using indices of the heavy body weight and appearance of urinary glucose [99]. Iizuka et al. [100] reported that TSOD mice develop mechanical hyperalgesia between six to twelve months old.

4) A very interesting model is the stress-induced T2DM mice model developed by Loizzo et al. [101], A post-natal psychological stress produced a series of dysmetabolic signs highly similar to mild human T2DM. Adult mice, receiving post-natal stress, display increased body weight, fasting glycaemia and increased plasma level of corticosterone and adrenocorticotrophic hormone (ACTH). Mice present thermal hyperalgesia in tail-flick test and administration of naloxone prevented body overweight and abdominal overweight suggesting an involvement of the opioid system and of the hypothalamus-pituitary axis. This model of stress should be useful to study idiopathic diabetes mellitus and neuropathy induced in these conditions [101].

5) STZ-induced T2DM model was developed by Srinivasan et al. [102] in order to replicate the natural history and the metabolic characteristics of human T2DM for suitable pharmacological screening. Authors used male *Sprague-Dawley* rats, which were fed with HFD (58% calories as fat), for a period of two weeks. HFD-fed rats exhibited significant increase in body weight, basal glycaemia, and insulinemia and also presented dyslipidemia. Then, rats received an intraperitoneal injection of a low dose of streptozotocin (35 mg/kg), which produces a decline of insulin secretion and transforms prediabetes status, induced by high fat feeding, in diabetes. Hence, rats present hyperglycemia, insulinopenia, insulin resistance, and dyslipidemia as patient with an advanced T2DM [102]. From this model Xiu-ying Yang et al. [103] showed that rats which received standard chow diet supplemented with 10% sucrose, 10% lard, 2% cholesterol and 0.2% cholic acid during one month followed by intraperitoneal injection of STZ (30 mg/kg) present thermal hypoalgesia and a decrease NCV which could be relieved by salivianolic acid A, an antioxidant [103].

All these models display painful thresholds changes; nevertheless, they are still marginal and most investigations will be necessary to improve their predictability in pain research.

Nowadays, T2DM rat or mice models are not systematically used for the study of DPN, probably because none of them is yet fully characterized but also because housing, maintain and using of knockout mice or congenic strains is more problematic than the use of the T1DM model induced by STZ which, contributed to most of our knowledge in DPN in the last thirty years.

Conclusion

Rodent models of T1DM and T2DM have vastly improved the understanding of pathophysiology of diabetic neuropathic pain and the development of new therapeutics. These models do not pretend to reproduce diabetic neuropathy, as it develops in humans, but to approach it, according to the "principle of similarity" defined by Bennett [104]. The conclusions from observations obtained in these models should be drawn with care and validated in more than one model or condition because diabetic patients with painful neuropathy come from a heterogeneous population in terms of etiopathogenesis, clinical course of diabetes and, for some of them, co-morbidities. Cancer is one example of co-morbidity and diabetes may negatively impact both cancer risk and outcomes of treatment. Indeed several chemotherapeutic agents like cisplatin, paclitaxel, and vincristine might cause or exacerbate neuropathy [105]. The deleterious effect of paclitaxel chemotherapy on thermal nociception was observed in STZ diabetic hyperglycemic rats [106] and further support the need for development of animal models closer to clinical reality.

This literature review reaffirms the need for collaboration between clinical and preclinical research to increase the benefit of pharmacological advances, and the relevance of the work on animal models.

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