Purinergic P2X receptors and diabetic neuropathic pain

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Abstract: Diabetic peripheral neuropathy (DPN), one of the most common chronic complications of diabetes, is characterized by allodynia, hyperalgesia and spontaneous pain. Chinese epidemiological studies have shown that at least 25% diabetic patients suffered from painful DPN, which compromises patients’ daily functioning and becomes a major health care problem. Although the pathogenesis of painful DPN is not fully understood and current treatment options are very limited, research in the field has advanced our understanding on the mechanism of painful DPN in the past Decade of Pain Research and Control. This review will mainly focus on evaluation of current diabetic animal models, possible molecular pathways and available therapies, with an emphasis on roles of purinergic receptor and its signaling transduction pathways. Common therapies address one or two DPN symptoms, while others offer wider symptom control, presumably by targeting pathophysiological mechanisms of DPN. Purinergic receptor signaling transduction pathways might become potential targets for treatment for painful DPN.

Key words: neuropathic pain; diabetes; P2X receptors; treatment

Diabetes mellitus (DM) is a debilitating chronic disease that affects ~8% of the population in the world. About 70% of diabetic patients in the United States are reported to have various types of nerve damage (neuropathy) [1]. The most common form of diabetic neuropathy is nerve damage in the periphery, such as hands, toes and
ankles, taking on distribution of gloves or socks. Diabetic peripheral neuropathy (DPN) patients often experience aberrant pain sensation, including spontaneous pain, hyperalgesia (severe pain with mild painful stimuli) and allodynia (pain with innocuous stimuli, e.g. light touch). Neuropathic pain (NeuP) is a pain arising as a direct consequence of a lesion or disease affecting the somatosensory system. As one of the most common NeuP, painful DPN in patients mainly involves lower limbs at the beginning: toes, feet and lower legs. The distal end of upper limbs can also be implicated at the later stage. The quality of pain is multifarious including burning, shooting, electric, knife cutting-like pain. The intensity and duration are varying within individuals. Patients may also have dysesthesias and paresthesias, such as crawling, itching, numbness, and tingling. When pain is induced by touch, allodynia, hyperalgesia and hypoaesthesia emerged [2]. As symptom severity increases, so too does the level of impairment in daily functioning and quality of life. Patients with severe DPN symptoms may experience quality-of-life impairments that are comparable to or even greater than those associated with functional gastrointestinal disorders or depression.

However, treatment options for these abnormal sensations have been limited, partly because of our poor understanding of pathophysiological mechanisms underlying the diabetes-induced neuropathic pain. The better treatment protocol for diabetic neuropathic pain should be based on the pathogenesis to explore and develop. Although development of pathophysiological and neuro-anatomical changes has been well described, the initial stage of painful DPN is not well understood. In the past several decades, especially in the past Decade of Pain Research and Control, research in the field has advanced our understanding on pathophysiological mechanism of painful DPN. To provide further detail, a comprehensive search of the literature was carried out for published basic and clinical studies. In this targeted review, we have summarized several manifestations emerged in painful diabetic models, with emphasis on current non-transgenic animal models. A large body of literatures has shown that these animals showed symptoms of mechanical hyperalgesia and allodynia. The advantages and disadvantages of different types of animal models are summarized in this paper. We then analyzed the possible molecular mechanisms involved in painful DPN. Sensitization of dorsal root ganglion neurons and their associated nerve fibers has been suggested to be a major cause of diabetes-induced abnormal pain. Changes in activities of pain related molecules in primary sensory neuron are discussed in this review [3,4]. Among them, P2X receptors are thought to contribute to changes in the activity of sensory neurons under diabetic conditions [5]. We focused on plasticity of P2X receptors and their possible transduction pathways. Last but not least, we assessed current clinical treatment options and proposed that purinergic receptor and its related signaling transduction pathways might become the potential target for treatment for painful DPN. After discussing current diabetic models and proposed molecular pathways underlying painful DPN, we hope to shed light on how to cure these patients who are tortured by painful DPN.

1 Experimental rodent models of diabetes

Enormous diabetic models have been established in the past several decades to explore molecular mechanisms of the complication of diabetes. These models can be generally classified into two categories: genetically induced spontaneous diabetes models and experimentally induced nonspontaneous diabetes models. The first type includes the Zucker diabetic fatty (ZDF) rats, Goto Kakizaki (GK) rats, spontaneously diabetic Tori (SDT) rats, Otsuka Long Evans Tokushima Fatty (OLETF) rats, Biobreeding (BB) rats, Diabetes (DB) mice, Obese (OB) mice, Nagoya Shibata Yasuda (NSY) mice and Toronto-KK (T-KK) mice. The second type is more widely used because of its lower construction cost, more simple administration and maintenance. Thus, we will mainly focus on this type in this review. This type of experimental diabetes can be conveniently induced by streptozotocin (STZ) or alloxan injection at neonatal or adult age. It can also be induced by high-fat diet, partial pancreatectomy, or combinations of these. Since these animals are not diabetic under normal conditions, thus this type of diabetes is termed experimentally induced diabetes models. Although there have been many successful diabetic models developed in nonhuman primates [6,7] or other mammals [8], they are less regularly employed because of high expense. In this section, we will focus on pros and cons of STZ-induced models, long-term high fat (HF) diet-fed models, and HF diet-fed plus STZ models (Table 1).

2 STZ-induced models

The first compound used for chemically induced diabe-
tes in animals is STZ or Alloxa which is a uric acid derivative \cite{9} and takes effect by means of selectively destroying pancreatic β-cells \cite{10}. Mechanical hyperalgesia has been mainly exhibited in STZ-\cite{11, 12} and alloxan-induced diabetic animals \cite{13–15}. This chemical is administered either by intraperitoneal (i.p.) or by intravenous (i.v.) injection through the tail vein of adult animals. For neonates, i.p. injection is a frequently used method. To date, multifarious doses have been injected into animals (35–90 mg/kg in rats or mice) \cite{16, 17}. Variations of blood glucose are closely related to the degree of β-cell damage in pancreas. Higher doses often induce more injuries of β-cell, thus inclined to induce T1DM. These models are characterized by progressively reduced pancreatic β-cell number, fasting or nonfasting hyperglycemia, lowered glucose-stimulated insulin secretion, and decreased glucose intolerance. The major features described in diabetes (polyphagia, polydipsia, polyuria) are conspicuous \cite{16–18}. The problem is that most of rats emerge a dramatic reduction in body weight and their health conditions are quite poor at later stages. Moreover, total cholesterol (TC) and triglyceride (TG) were not altered in this model. Thus, other types of experimentally induced diabetic animal models are developed.

3 Long-term high fat (HF) diet-fed models

Diet-induced diabetes is a classical process to T2DM. It contains most peculiar characteristics of T2DM in patients, including insulin resistance, obesity, weight gain, hyperinsulinemia and increased serum total TC levels \cite{19–22}. A major limitation to this approach is the time required for induction of diabetes. As we know, obesity does not result in a distinct diabetic profile itself in the short time. Therefore, one routinely address-

es this type as long-term high-fat diet models. Among numerous models of diabetes, C57BL/6J mice are manifested to display greater sensitivity to HF-diet (simple carbohydrate) \cite{9}. HF diet rat models have also been described \cite{23}. Rats fed with HF diet after at least 8 weeks develop T2DM.

4 HF diet-fed plus STZ models

To integrate the merits and demerits of the two types above, another model-HF diet-fed plus STZ rat/mouse models appeared. They are first reported by Hutton et al. in 1976 \cite{24}. By using a combination of an HF diet and a chemical inducer, diabetes can be induced more quickly. With respect to the induction of model, diversified methods have been employed. Some contain higher fat diet-fed and lower-dose STZ, such as 30% HF diet-fed followed with STZ (15 mg/kg) \cite{25}. Due to lower dose of STZ, it takes a longer time to develop diabetes. These animals display hyperglycemia, hyperinsulinemia, impaired glucose tolerance, insulin resistance and dyslipidemia. Another method involves lower fat diet-fed and higher-dose STZ. For example, 7% HF diet-fed accompanied with STZ (65 mg/kg) i.p. injection \cite{26}. It takes relatively shorter time to induce hyperglycemia, hyperinsulinemia, moderate insulin resistance, dyslipidemia and increased liver glycogen levels in these animals. Undoubtedly, TC and low density lipoprotein-cholesterol (LDL-C) levels are not as high as those with higher HF diet. The third model includes higher HF diet-fed plus higher-dose of STZ. The first researcher described a T2DM model induced by feeding a diet containing 40% fats of the total calories originally, followed by an i.p. injection of STZ (50 mg/kg). Noticeably, these models produced increased body weight, fasting blood glucose, TG and free fatty acid

<table>
<thead>
<tr>
<th>Model</th>
<th>β Cell</th>
<th>Insulin content</th>
<th>Hyperglycemia</th>
<th>G. I.</th>
<th>Polyphagia</th>
<th>Insulin resistance</th>
<th>BW</th>
<th>TC</th>
<th>TG</th>
<th>Time needed</th>
<th>Reference</th>
</tr>
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<tr>
<td>Adult/ neonatal STZ</td>
<td>↓</td>
<td>↓</td>
<td>Yes</td>
<td>↓</td>
<td>Yes</td>
<td>No</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>Short</td>
<td>\cite{6, 9–14}</td>
</tr>
<tr>
<td>Long-term HF diet-fed</td>
<td>↑</td>
<td>Yes</td>
<td>Yes</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Long</td>
<td>\cite{5, 15–19}</td>
<td></td>
<td></td>
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<tr>
<td>HF diet-fed STZ</td>
<td>↓/↑</td>
<td>Yes</td>
<td>↓</td>
<td>Yes</td>
<td>↓/↑</td>
<td>↑</td>
<td>Long</td>
<td>\cite{21, 22}</td>
<td></td>
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<tr>
<td>Partial pancreatectomy</td>
<td>↓</td>
<td>Yes</td>
<td>No</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>Short</td>
<td>\cite{25–28}</td>
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(FFA) levels. The remaining models are induced with moderate HF diet-fed and moderate dose of STZ. Amid those, it is concluded that the models of a 20% HF diet-fed plus 40 mg/kg STZ is the optimized combination. The use of HF diets combined with STZ has also been applied to mice, such as C57BL/6J mice and ICR mice. ICR mice are more susceptible to the effects of HF diets and STZ administration.

Other models including partial pancreatectomy are also characterized by hyperglycemia and reduced β-cell numbers, decreased levels of serum and pancreatic insulin and insulin resistance. The problem for pancreatectomy is that the residual pancreatic β-cells have a strong capability of regeneration. This could be a primary restriction for use of this model. Paralleling with STZ model, this type of model will lose weight and the major feature of T2DM-insulin resistance could not be well shown.

As discussed above, several models have been established in the past several decades. Merits and demerits of these models are summarized in Table 1. With regard to STZ-induced models, they are not always a good representation of human diabetes. Since it is easily manipulated and takes short time to induce, this model is widely used recently. Long-term HF diet-fed models have more realistic significance. However, they need long time to develop and will cause high expense. Accordingly, a combination of HF diet-fed and STZ-induced models appeared. This model takes advantages of both models and excludes their disadvantages. Therefore, they might be one of the best models suitable for the study of mechanisms of neuropathic pain in DM.

5 Quantitative assessment of mechanical pain sensation and tactile allodynia

It has been reported that ~70% of painful DPN patients expressed mechanical hyperalgesia in early PDN, while patients in advanced PDN expressed hypoalgesia. The von Frey filament (VFF) method is the most frequently used approach to quantitate the pain behavioral. It is composed of several filaments representing a series of calibrated filaments. Rats/mice are placed in plastic cages with a wire mesh floor which allowed full access to the paws. Pricking area is the plantar surface of hindpaw without fur. Paw withdrawal, paw raising and hissing are regarded as positive reactions. Voluntary movement associated with locomotion is not considered to be a withdrawal response. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the “up-down” method. In the presence of a response, the filament of next lower force was applied. In the absence of a response, the filament of next greater force was applied. Each filament is applied five times at 30-s intervals to the hindpaw, and the response threshold is defined as the lowest force that caused at least three withdrawals of the five consecutive applications. The von Frey aesthesiometer electronic version is more simple and convenient, which is consisted of a hand-held force transducer fitted with a 0.5–0.7 mm² polypropylene tip. Pricking area and positive reaction are the same as conventional VFF. More preferably, the intensity of the pressure can be recorded automatically after the paw withdrawal.

Randall-Selitto pressure test is another method often used to determine pain threshold of rat paw and mouse tail. Pressure increases at a linear rate of 10 g/s, with the cutoff of 250 g to avoid tissue injury. The pressure is applied to the center of rat hindpaw or the base of mouse tail. The applied pressure is registered by an algnesia meter and expressed in grams. Intensity of pressure to cause an escape response is defined as withdrawal threshold. Several tests separated by at least 15 min are performed for each animal and the mean value of these tests is calculated as threshold.

There exists another painful diabetic neuropathy-tactile allodynia. It is a painful perception of a light touch (in response to nonpainful stimuli) if threshold is calculated as <4 g based on prior comparisons with normal rats. Tactile allodynia develops quickly after STZ injection and appears to involve a mechanism different from that underlying mechanical hyperalgesia. As the change in withdrawal threshold measured by Randall-Selitto test may be the results of cutaneous mechanical hyperalgesia, flexible VFF are also regular methods to be used for quantitative assessment of tactile allodynia. Recently, we showed that STZ administration significantly reduced the paw withdrawal thresholds both in male and female rats. This reduction, however, was greater in female rats (2–3 g) than in male rats (5–6 g). It is, thus, of interest to know whether there is a sex difference of mechanical pain thresholds between male and female animals and mechanisms involved after induction of diabetes.
6 Plasticity of P2X receptors and painful DPN

Pathogenesis of painful diabetic polyneuropathy is poorly understood. While painful DPN is generally thought to be associated with sensitization of peripheral neurons, no consensus definition exists on molecules of responsible for these well-recognized abnormalities. However, recent studies have proposed that several signaling pathways may participate in the process of diabetic pain. These include PKC-mediated TRPV1 sensitization [46–48], hyperglycemia-mediated oxidative stress [49–52], and changes in plasticity of purinergic receptors. In this review, we mainly focus on plasticity of P2 receptors and their signal transduction pathways. Of course, we do not exclude contributions from other molecules, which might also play an important role in the development of painful DPN. However, the key role of P2 receptors and their signal transduction pathways in painful DPN has given rise to the hypothesis that altered purinergic signaling leads to either hyperalgesia or allodynia.

7 P2X3 receptors in sensory neurons

According to molecular structure and mechanism of signal transduction, P2 receptors are divided into two subtypes: P2X and P2Y. P2X receptor is a ligand-gated cation channel generating inward current evoked by ATP [53], while P2Y receptor belongs to G-protein coupled receptor family [54]. In 1983, Krishtal et al. firstly confirmed the existence of ATP receptors on dorsal root ganglion (DRG) [55]. In the primary sensory nervous system, the particularly noticeable is P2X3 receptor, which is highly selectively expressed in small diameter DRG neurons relative to nociception. Much information has showed that DRG neurons dominated in skin and viscera express P2X3R for 30%–40%, while there are only 2% DRG neurons dominated in skeletal muscles [56]. ATP is not only an important energy molecule, but also a cell-to-cell information transfer mediator. It is released from damaged cells as a result of inflammation or injuries. It plays a mediator role through cell surface P2 receptors [57]. Because ATP is released from peripheral tissues, including skin, P2X3 receptors in the periphery could be involved [58]. P2X3 receptor-mediated responses are greatly enhanced under abnormal pain, such as hyperalgesia and allodynia [59, 60]. Studying the involvement of P2XRs in painful diabetic neuropathy, Migita et al. and our lab found that P2X receptor antagonists inhibit the STZ-induced mechanical allodynia in mice [5] and in rats [1], respectively. The molecular mechanism underlying the change in P2XR-mediated activity, however, has not been fully investigated. Migita et al.[5] found that the levels of P2X2R and P2X3R mRNA in DRGs were increased following STZ-induced diabetes. They did not examine the expression of this receptor at protein levels under this condition. We recently reported that there was a significant upregulation of membrane P2X3R protein expression without alteration in expression of total P2X3R protein in rat DRGs, indicating that the trafficking of P2X3R from cytoplasm to cell surface membrane was enhanced under diabetic neuropathic conditions. The increase (85.7%) in P2X3R trafficking would partially account for the large (2.2-fold) increase in P2X3R-mediated currents following STZ-induced diabetes.

The mechanism underlying the increase in trafficking of P2X3Rs following diabetes has yet to be determined. In an effort to determine the relationship between P2X3 receptors and calcium/calmodulin-dependent protein kinase II (CaMKII) in DRG neurons, we found that electrical stimulation-activated CaMKII promotes membrane expression of P2X3 receptors [61]. CaMKII is a kind of serine threonine kinases. They are located in 45% of rat DRG neurons and most of them are involved in the processing of nociceptive information [62]. After inflammation and nerve injuries, CaMKII expression is enhanced [63]. Although it is not clear whether CaMKII activity is altered in DRG neurons, there is growing body of evidence that high phosphorylated levels of CaMKII were reported in the diabetic rat heart papillary muscles [64] and in the diabetic retinas [65, 66]. In addition, results from the in vitro experiments indicate P2X receptors can be differentially recruited to specific membrane domains of lens fiber cells by osmotic and hyperglycemic stress; In response to hypertonic stress P2X (1) and P2X (4) isoforms became more closely associated with the broad sides of fiber cells, while under hypotonic conditions P2X (4) and P2X (6) isoforms associate with the narrow side membranes. No such changes in subcellular distribution were observed for P2X (2, 3 and 7) isoforms [67]. Rat retinal neural cells cultured in high glucose conditions show increased calcium responses to P2 receptors activation [68]. Together, these data show that high glucose-induced changes in P2X3 receptor plasticity is involved in diabetic neuropathy (Fig. 1).
8 Other P2X receptors in glial cells

In addition to P2X3R, an increasing body of evidence revealed that P2X4R and P2X7R are involved in diabetic neuropathy. In the spinal cord, expression of P2X4R is enhanced and is highly restricted to microglia which are activated after nerve injury. P2X7 was required for T1D acceleration induced by CD38 deficiency. The detailed mechanisms are unknown. One possibility is that activation of P2X4R evokes a rise in intracellular Ca\(^{2+}\) level that leads to activation of intracellular Ca\(^{2+}\)-sensitive signaling cascades, such as p38 mitogen-activated protein kinases.

9 Potential role for hydrogen sulfide (H\(_2\)S) in P2X receptor plasticity

H\(_2\)S, a gas synthesized by sulfate reducing colonic bacteria and the endogenous enzymes cystathionine-\(\beta\)-synthetase (CBS) and cystathionine-\(\gamma\)-lyase (CSE), is increasingly recognized as a biologically important signaling molecule in various tissues and processes including pain and inflammation. There is evidence that intraplantar injection of NaH\(_2\)S (a commonly used H\(_2\)S donor) into rat hindpaws produces mechanical hyperalgesia. Further, H\(_2\)S generation is also enhanced in the formalin, carrageenan and complete Freund’s Adjuvant-induced model of persistent inflammatory pain, supporting a pro-nociceptive role for H\(_2\)S. Recently, we have showed that CBS expression was significantly enhanced in DRGs of STZ-induced diabetic pain rats (our unpublished observations). It is, therefore, reasonable to hypothesize that CBS-H\(_2\)S signaling pathway might be involved in diabetic neuropathic pain. The enhanced expression of CBS leads to an increase in H\(_2\)S production in the DRG. H\(_2\)S has been reported to modulate various ion channels including voltage-gated calcium channels and ligand-gated receptor channels. H\(_2\)S also increases the influx of calcium ions. As we reported previously, increase in intracellular calcium concentration activates CaMKII, which promotes the trafficking of P2X3 receptor from cytoplasmic vesicles to cell membrane (Fig. 1). In addition, we have reported that CBS and P2X3R are colocalized in DRG neurons. Together, these data suggest that the CBS-H\(_2\)S signaling pathway plays an important role in painful DPN. Further experiments are needed to determine the role for CBS-H\(_2\)S signaling pathway in P2X receptor-mediated diabetic neuropathic pain.

10 Managements of diabetic neuropathic pain

Treating painful DPN remains a significant clinical challenge, and in particular current treatment options are very limited and marginally effective. There is agreement that treatment is on the basis of four pillar stones: (1) causal treatment aimed at (near)-normoglycemia; (2) treatment based on pathogenetic mechanisms; (3) symptomatic treatment; (4) avoidance of risk factors and complications. Currently, there are no ideal therapies. Two principles were summarized here: etiological treatment and treatment for improvement of pain symptoms. From the clinical point of view, treatments due to pathogenesis of disease are the most fundamental and appropriate methods.

**Conventional therapies:** Tricyclic antidepressants (TCAs) are regarded as most studied drugs aiming at neuropathic pain. TCAs appear to improve global DPN symptoms but have variable effects on uncertain tolerability. The most frequent adverse events of TCAs include tiredness and dry mouth. The starting dose should be 25 mg (10 mg in frail patients) and taken as a single night time dose one hour before sleep. It should be increased by 25 mg at weekly intervals until pain relief is achieved or adverse events occur. The maximum dose is usually 150 mg per day. Although they are not in...
connection with mechanisms of neuropathy and can’t alter natural course of the disease, they can improve patient’s quality of life. The possible mechanism of action is that TCAs inhibit the ingestion of norepinephrine and serotonin by neuronal synapsis via suppressing sodium channels. Thus, the pain threshold is raised and generation of nerve impulses by impaired nerves is prevented. Among TCAs, amitriptyline and imipramine are the most usual drugs for neuropathic pain. It is demonstrated that adenosine receptors are involved in the anti-allodynic effect of amitriptyline in STZ-induced diabetic rats [80].

Patients have failed to respond to TCAs or are taboo to TCAs, anticonvulsant should be considered. Antiepileptic drugs (AEDs) have a long history of effectiveness in the treatment for neuropathic pain [81]. Their primary mechanisms of action involve calcium and sodium channels blockade, antagonism of glutamate at N-methyl-D-aspartate receptors [82, 83] and intensification of γ-aminobutyric acid (GABA) activity [84]. These pharmacological actions can be combined for synergistic effect. Currently, Food and Drug Administration (FDA) only recognizes pregabalin and duloxetine as specialized drugs for diabetic neuropathy. Gabapentin is a novel anticonvulsant drug, which is a derivative of GABA. Compared with other AEDs, such as dilantin sodium and carbamazepine, gabapentin has smaller behavioral and cardiovascular side effects.

When coupled with severe pain and resistant to the drugs above, patients can choose to use opiates. The intensity of pain is confronted by endogenous analgesic mediators involving opioid peptides [85]. Opioid peptides were found both in monocytes and neutrophils. They can be triggered by chemokines or hormones thereby release and bind to opioid receptors on peripheral sensory neurons [86, 87], thus produce an analgesia effect. When spinal opioid receptors are blocked, subsequent systemic administration of opioids fails to produce an analgesic effect, which highlights the important role of opioid receptors in the spinal cord in the antinociceptive action of opioids [88]. It has also been reported that electroacupuncture (EA) stimulation reduces inflammatory pain through activation of mu-opioid receptors [89]. EA just increases endogenous opioid peptides and promotes insulin secretion, inducing a hypoglycemic response in animals [90]. For example, tramadol, 200 mg qd, can result in a beneficial effect. Recently, as a conventional drug for rehabilitation, methadone receives a wide publicity. Although lacking of evidence about clinical trial, methadone is effective to neuropathic pain.

Targeted therapies: In contrast with conventional therapies, which treat only one specific symptom of DPN, targeted treatment strategies are directed at addressing the underlying pathophysiological mechanisms that are believed to cause DPN. As we discussed above, adenosine, 5’-triphosphate (ATP) is a ubiquitous energy donor and receptor ligand in living cells in the millimolar concentration range and released into the extracellular milieu after tissue injury or inflammation. Once released, it activates ATP receptors on nearby sensory nerves. Their activation by ATP induces the opening of a pore permeable to Na⁺, K⁺ and Ca²⁺, leading to an overall depolarization of the cell [91]. A boost to the field has derived from the use of genetic methods such as knockout mice and antisense oligonucleotides, as well as the availability of P2X receptor specific antagonists. P2X3 receptor gene deletion results in a significantly attenuated nociceptive phenotype in mice, including altered sensitivity to thermal stimuli and decreased pain-related behaviors in animals with carrageenan or formalin-induced inflammation [92], and altered function of visceral organs, such as the urinary bladder and small intestine [93, 94]. Reduction of P2X3 receptor expression via intrathecal administration of P2X3-selective antisense or siRNA also leads to a marked decrease in behavioral responses of chronic inflammatory and neuropathic pain in mice. These data suggest that P2X receptor gene deletion might be a potential tool for treatment of painful DPN. Pharmacological studies linking P2X3 receptors with nociception showed that P2X receptor antagonists inhibit the STZ-induced mechanical allodynia in mice [5] and rats [1]. These results indicate that antagonisms of P2X receptors would be another tool for treatment of painful DPN. However, these studies relied on the use of chemical molecules with poor potency, selectivity and permeability, such as pyridoxal phosphate-6-azo (benzene-2,4-disulphonic acid), reactive blue 2, suramin, and/or low metabolic stability, such as 2',3'-O-(2,4,6-trinitrophenyl) ATP, making them less than ideal for in vivo experiments [95–97]. Several groups have developed chimeric P2X protein subunits. A-317491, a more selective and potent compound with low molecular weight, was described by Jarvis et al [94]. However, undesirable futures, including very high protein binding (>99%), low oral bioavailability and poor CNS penetration, also limit its use as an in vivo tool com-
compound. Most recently, AF-353, a compound with unique chemical structure, demonstrated high antagonist potency at and dual selectivity for P2X3 and P2X2/3 channels, moderate protein binding, oral bioavailability and attractive pharmacokinetic profile suitable for in vivo studies \cite{98}. However, these newly developed compounds have not been fully investigated in an animal model of painful DPN.

11 Future directions and conclusion remarks

An emerging theme that unifies many supposedly diverse painful DPN is altered neuronal excitability, caused by abnormal expression and function of membrane channels in organ-specific primary sensory neurons. As discussed above, P2X receptors play an important role in painful DPN. Purinergic receptors as the main determinants of intrinsic neuronal excitability are particularly appealing targets for pharmacological intervention. Other ion channels such as vanilloid receptor, ion channels, which are certainly involved in pain processing, have not been discussed in this review. Nevertheless, the advent of new experimental medicine techniques presents us with an opportunity to investigate the effectiveness of novel medicines in great detail. The ability to perform hypothesis-driven research with tool compounds in diabetic patients with demonstrable hypersensitivity is a stimulating prospect. For example, a drug that selectively inhibits the purinergic subtype receptors, which appears to be crucially and specifically involved in visceral nociception, would presumably act as a novel and powerful analgesic, with few side effects in most indications of diabetes-related visceral complication. Although some compounds with desirable characteristics, including high antagonist potency and dual selectivity for P2X3 and P2X2/3 channels, moderate protein binding, oral bioavailability and attractive pharmacokinetic profile have been developed in the past couple of decades \cite{98}, they are not tested in an animal model of painful DPN yet. It is of great interest.

In conclusion, painful DPN is an important subject for both researchers and clinicians. Although considerable progresses have been made in the past decades, the underlying mechanisms and effective treatments are still awkward problems. In the future, painful DPN is definitely an important subject and much more effort should bring more gospels for diabetic patients. Depending on the nature of the symptoms in the individual patient with diabetes, each of these targeted therapies should be able to provide benefit that goes beyond the mono-symptomatic relief conferred by conventional therapies. Knowledge of the differential treatment effects of each of these agents may enable a more personalized treatment approach in diabetes. The latest data suggest that whereas acute pain seems to be linked to the activation of P2X3 receptors expressed in sensory neurons, neuropathic pain more likely involves P2X4 receptors on the surfaces of glial cells, and P2X7 receptors on immune cells in type 1 diabetes \cite{70}. As new therapies are investigated, the effects of specific agents on multitiered patient-reported outcome measures will be informative to the field and will help shape future evidence-based practice guidelines for the treatment of painful DPN.

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