Journal of Pain Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

K_v7 Channel Activators Flupirtine and ML213 Alleviate Neuropathic Pain Behavior in the Streptozotocin Rat Model of Diabetic Neuropathy

Ashraf Ibrahim Ahmed[1], Salma Al-Nuaimi[1], Ayman Mustafa[1], Asad Zeidan[1], Abdelali Agouni[2], Laiche Djouhri[1]

[1]Department of Basic Medical Science, College of Medicine, QU Health, Qatar University, Doha, Qatar; [2]Department of Pharmaceutical Sciences, College of Pharmacy, QU health, Qatar University, Doha, Qatar

Correspondence: Laiche Djouhri, Email ldjouhri@qu.edu.qa

Background & Objective: Chronic peripheral neuropathic pain (PNP) is a debilitating condition that is associated with many types of injury/diseases, including diabetes mellitus. Patients with longstanding diabetes develop diabetic PNP (DPNP), which is resilient to currently available drugs. The underlying molecular mechanisms of DPNP are still illusive, but K_v7 channels that have been implicated in the pathogenesis of various types of chronic pain are likely to be involved. Indeed, using the streptozotocin (STZ) rat model of DPNP, we have previously shown that K_v7 activation with their non-selective activator retigabine attenuated neuropathic pain behavior suggesting that these channels are implicated in DPNP pathogenesis. Here, we evaluated, in the same STZ model, whether the more potent and more selective K_v7 channel openers flupirtine and ML213 attenuate STZ-induced pain hypersensitivity.

Methods: Male Sprague Dawley rats (250–300 g) were used. The STZ model involved a single injection of STZ (60 mg/kg, i.p.). Behavioral testing for mechanical and heat pain sensitivity was performed using a dynamic plantar aesthesiometer and Hargreaves analgesiometer, respectively.

Results: STZ rats exhibited behavioral signs of mechanical and heat hypersensitivity as indicated by significant decreases in the mean paw withdrawal threshold (PWT) and mean paw withdrawal latency (PWL), respectively, at 35 days post-STZ treatment. Single injections of flupirtine (10 mg/kg, i.p.) and ML213 (5 mg/kg, i.p.) to STZ rats (35-days after STZ treatment) caused significant increases in the mean PWT, but not PWL, indicating attenuation of mechanical, but not heat hypersensitivity. Both flupirtine and ML213 were as effective as the positive control gabapentin (10/kg, i.p.), and their anti-allodynic effects were prevented by the K_v7 channel-specific blocker XE991 (3 mg/kg, i.p.).

Conclusion: The findings suggest that K_v7 channels are involved in the mechanisms of mechanical but not heat hypersensitivity associated with DPNP, and that their activation may prove to be effective in alleviating DPNP symptoms.

Keywords: ion channels, diabetic neuropathy, chronic pain, rat model of pain, K_v7 channels

Introduction

Pain is "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" according to the International Association for the Study of Pain (IASP). Chronic peripheral neuropathic pain (PNP), pain caused by a lesion or disease of the somatosensory nervous system, is a debilitating condition that is associated with many types of injury/diseases, including diabetes mellitus that affects hundreds of millions of people worldwide. Up to 50% of patients with longstanding diabetes develop diabetic PNP (DPNP).[1] It is estimated that, without successful intervention, about one-third of the expected 9.7 billion people living in 2050 will have diabetes and about 50% of those will have DPNP.[2] It remains unclear why only some patients with diabetic neuropathy develop DPNP (see[3]). Patients with DPNP usually experience a range of unpleasant symptoms including spontaneous burning pain, pain hypersensitivity to normally noxious and innocuous stimuli (hyperalgesia and allodynia) and dysesthesias/paresthesias (see e.g.,[3]) Successful therapy for PNP remains a challenge because

Received: 6 March 2024
Accepted: 18 June 2024
Published: 26 June 2024

currently available drugs are either ineffective and/or have adverse side effects. Indeed, only about 30% of PNP patients experience a pain reduction of about 30%.[4]

The pathogenesis of DPNP is still illusive, but several mechanisms have been proposed including hyperexcitability of dorsal root ganglion (DRG) neurons (e.g.[5]). Indeed, when injured, these sensory neurons become hyperexcitable and start generating abnormal spontaneous nerve impulses/action potentials (APs) and develop an altered stimulus–response function (e.g.[6–8]), though it is unclear how diabetes targets and injures these sensory neurons.[3] Using rodent models of DPNP, we and others have previously shown that DRG neurons exhibit spontaneous APs, the key characteristic of neuronal hyperexcitability.[9–12] This aberrant spontaneous activity is believed to be crucial for initiation and/or maintenance of PNP and to drive the excitability changes in CNS, another important PNP mechanism.[13]

The molecular mechanisms underlying the abnormal hyperexcitability of DRG neurons associated with DPNP are incompletely understood. However, changes in expression and/or activation properties of neuronal voltage-gated K_v7 ($K_v7.2$–7.5) channels in these neurons (during chronic pain states) could result in membrane depolarization, and render them more prone to abnormal AP firing. This is because these channels underlie the outward current (I_M) that normally exerts a powerful stabilizing influence on neuronal excitability.[14] Using the streptozotocin (STZ) rat model of DPNP, we have previously shown that activation of K_v7 channels with retigabine alleviates STZ-induced pain behavior.[15] However, this anticonvulsant does not show selectivity for any particular neuronal K_v7 channel subtypes, and shows unspecific effects on other targets like GABA receptors.[16] Therefore, the present study was aimed at examining, in the same STZ model, whether the more potent and selective K_v7 channel openers flupirtine and ML213 attenuate STZ-induced pain hypersensitivity. Our results show, for the first time, that these K_v7 channel activators were effective in alleviating neuropathic behavior in the STZ model of neuropathic pain. Our results are consistent with our previous findings[15] that activation of K_v7 channels with retigabine dose dependently attenuated mechanical, but not heat hypersensitivity in STZ rats.

Materials and Methods

Experimental Animals

A total of 66 male Sprague Dawley rats (250–300 g) were used in the present study. They were housed four rats per cage in a room in the animal house (LARC) of Qatar University (QU). The room was kept at room temperature between 21°C and 24°C with 12-h light/dark cycles and free access to food and water. The experimental protocols were approved by QU ethical review committee (IACUC) and complied throughout with the UK Animals (Scientific Procedures) Act 1986.

The Streptozotocin (STZ) Rat Model of Diabetic Peripheral Neuropathic Pain (DPNP)

A few rodent models of DPNP have been developed to investigate its pathophysiology, including the widely used STZ rat model (see e.g.[17]). We used the STZ model because it is more commonly used than other models of diabetes mellitus and because of its rapid induction, greater stability and low cost as reported previously.[12,15,18] It involves a single injection of STZ (60 mg/kg, i.p.) after an overnight fast to reduce competition between STZ and glucose for uptake into pancreatic β-cells. STZ rats show a significant elevation in blood glucose as early as 3 days post-STZ (see[15]).

Pain Behavioral Testing

All the rats underwent pain behavioral testing. The testing was performed in plastic chambers after the rats had been acclimatized for 5–7 consecutive days to the procedure room and the testing chambers used for assessing mechanical and heat sensitivity as described previously.[15] The rats were also habituated to the chambers for at least 15 min before each testing. After the habituation to the chambers and after the exploratory and grooming behavior had ceased, mechanical and heat pain sensitivity of the left hind paw of each rat was assessed by measuring the paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) to mechanical and heat stimuli respectively as described below.

Behavioral Testing for Mechanical Pain Sensitivity

As described previously,[15] behavioral testing for mechanical pain sensitivity is performed using a dynamic plantar aesthesiometer touch stimulator (Basile, Italy), an automated von Frey type system. Briefly, rats are placed in plastic

boxes/chambers (15x15x20 cm) on a wire mesh. Then a blunt metal probe (0.5 mm in diameter) is used to stimulate the mid-plantar surface of the left hind paw of each rat three times (with a minimum of 5-min interval between each trial). An abrupt withdrawal of the hind paw in response to the mechanical force (applied with the metal probe) is taken as a positive response. The average force (in grams) of the three trials that result in paw withdrawal is calculated and considered as PWT. The baseline values are obtained from pain behavioral testing performed one day before STZ injection (pre-STZ). To prevent injury of the hind paw by the mechanical stimuli applied by the metal probe, a cutoff of 50 g was used. For each STZ rat, the behavioral pain testing is repeated at 21, 28 and 35-days post-STZ to determine whether the rats developed mechanical hypersensitivity/allodynia. Mechanical hypersensitivity is indicated by a significant decrease in the mean PWT as reported previously (e.g.[15]). The analgesic effects of drugs are determined from behavioral pain testing conducted 35-days post-STZ at 1–2 hours and 24 hours after drug treatment.

Behavioral Testing for Heat Pain Sensitivity

Behavioral testing for heat pain sensitivity is performed using Hargreaves analgesiometer (Ugo Basile, Comerio, Italy). The procedure in this test is similar to that used for mechanical hypersensitivity (see above) except that rats are placed on a glass floor (2-mm-thick) under which a movable infrared (laser radiant heat) source is positioned by the operator directly beneath the hind paw. A trial is started by pressing a key on the infrared source which activates a reaction time counter that stops automatically when paw withdrawal is detected by a photocell of the analgesiometer. The withdrawal heat latency to the nearest 0.1 s is automatically determined and recorded. The average response latency (in seconds) of three trials (with a minimum of 5-min interval between each trial) is calculated and considered as paw withdrawal latency (PWL). To prevent hind paw from tissue injury by the heat stimuli, a cutoff of 20 s was used. Heat hypersensitivity is indicated by a significant decrease in the mean PWL to the noxious heat stimulus applied to the midplantar surface of the hind paw (L4 dermatome) of each rat as described previously (e.g.)[15]

Drugs and Animal Groups

Of the 66 rats used in the present study, 54 rats received a single injection of a STZ (60 mg/kg, i.p.) referred to STZ-rats throughout. The remaining 12 rats were not treated with STZ (naïve) and were used for examining the effects of the K_v7 agonists flupirtine (10 mg/kg, n=6) and ML213 (5 mg/kg, n=6) on baseline pain sensitivity (see Figure 1). The STZ-rats (n=54) were divided into six groups: (1) group 1 (flupirtine group, n=10); each rat in this group received a single dose of flupirtine (10 mg/kg), (2) group 2 (ML213 group, n=10 rats); each rat in this group received a single dose of ML213 (5 mg/kg), (3) group 3 (gabapentin, positive control group, n=10); each rat in this group received a single injection of gabapentin (10 mg/kg), (4) group 4 (EX991 + flupirtine, n=8); each rat in this group received a single dose of the K_v7 antagonist EX991 (3 mg/kg) plus a single dose of 10 mg/kg flupirtine, (5) group 5 (EX991 + ML213, n=8); each rat in this group received a single dose of the Kv7 antagonist EX991 (3 mg/kg) plus a single dose of 5 mg/kg ML213, and (6) vehicle control group (n=8); each rat in this group received 1mL/kg of the vehicle (DMSO dissolved in physiological saline). Drugs (Sigma-Aldrich, St. Louis, MO) were dissolved in dimethyl sulphoxide (DMSO). All the aforementioned drugs and the vehicle were administered intraperitoneally (i.p.) at a volume of 1 mL/kg.

The dose of flupirtine (10mg/kg) used in the present study is based on a previous study[19] that examined the antinociceptive effects of three doses of flupirtine (7.5, 10 and 20 mg/kg, i.p.) in a rat model of gout arthritis pain, and found that flupirtine significantly attenuated mechanical and heat hypersensitivity in a dose-dependent manner. As for ML213, there is very little information about its in vivo effects in rodents. Indeed, its analgesic capacity has not been explored until now and, as far as we know, there is only one report about its effects in vivo.[20] These investigators examined effects of different doses of ML213 (1, 5, 10, and 20 mg/kg (i.p.) on locomotor behavior in rats, and found a significant effect at the 5, 10, and 20 mg/kg doses. Therefore, we used the lowest effective dose of 5 mg/kg in the present study. It should be noted that ML213 has been shown to act more specifically on K_v7 channels and to be more potent than retigabine (see Discussion). ML213 was originally reported as a selective $K_v7.2$ and $K_v7.4$ channel opener,[21,22] but subsequent studies showed that it is a stronger activator of $K_v7.3$ than retigabine[23] and a potent agonist for Kv7.5 channel.[24]

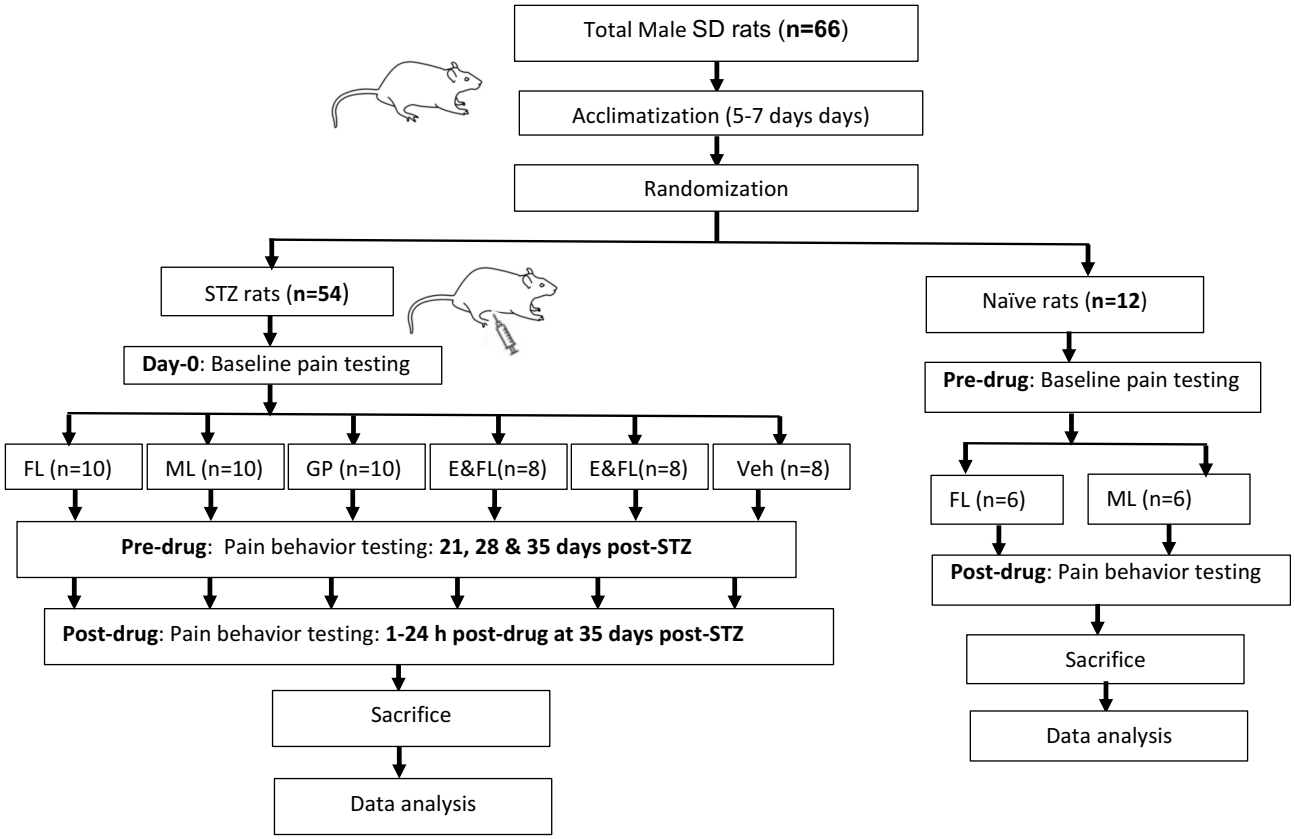

Figure 1 A schematic representation of the experimental design and the timeline of the behavioral pain testing. Of the 66 male SD (Sprague Dawley) rats used, 54 were STZ rats (received a single injection of 60 mg/kg, i.p.) and the remaining 12 rats were naïve (not treated with STZ). All the rats were acclimatized/habituated for 5–7 consecutive days to the procedure room and the testing chambers. After acclimatization, the rats were randomly divided into STZ and naïve rats. The STZ rats were divided into six groups: (1) FL (flupirtine group, n=10), (2) ML (ML213 group, n =10 rats), (3) GP (gabapentin, positive control group, n=10), (4) E & FL (EX991 plus flupirtine, n=8), (5) E & ML (EX991 plus ML213, n=8) and (6) Veh (vehicle control group (n=8). Pain behavioral testing was conducted before STZ treatment (baseline pain testing) and 21, 28 and 35 days post-STZ as well as 1–24 hours post-drug (FL and ML) at 35-days post-STZ. For the naïve rats, pain behavioral testing was conducted before (pe-drug) and after (post-drug) FL and ML administration.

Statistics Analysis

The data are presented as mean ± SEM because they were normality distributed (Shapiro–Wilk normality test). Comparisons between the mean pre-drug values of PWT and PWL and post-drug values were made using one-way repeated-measures analysis of variance (ANOVA) followed with Tukey's multiple comparison (post hoc) test. Statistical tests were performed using Graphpad Prism 10.2 (Graphpad Software, San Diego, CA). P values of less than 0.05 were considered significant and the level of statistical significance is indicated on the graphs with asterisks as follows: **p < 0.01; and * < 0.05.

Results

STZ Rats Exhibit Behavioral Signs of Both Mechanical and Heat Hypersensitivity

In order to examine the analgesic ability of the K_v7 channel openers flupirtine and ML213 in STZ rats, we conducted, in STZ-rats, behavioral testing for mechanical and heat pain sensitivity at 3, 4 and 5 weeks post-STZ to confirm that the STZ rats develop mechanical and heat hypersensitivity reported previously (e.g.[15]). The values of PWL and PWT at these time points which are based on our previous studies (e.g.,[15]) were compared with the baseline (pre-STZ) values. STZ treatment significantly decreased the mean PWL and the mean PWT (Figure 2A and B). As shown in Figure 2A, the decrease in PWL was highly significant (p <0.01) at 21, 28 and 35 days post-STZ indicating development of heat hypersensitivity at these time points. However, the decrease in the PWT (Figure 2B) was significant (p < 0.01) only at 35-day time point indicting a delay in the development of mechanical hypersensitivity. Overall these findings are in general agreement with those of our previous studies.[15] However, the STZ-induced decreases (% decrease) in both PWT and PWL found in the present study are lower

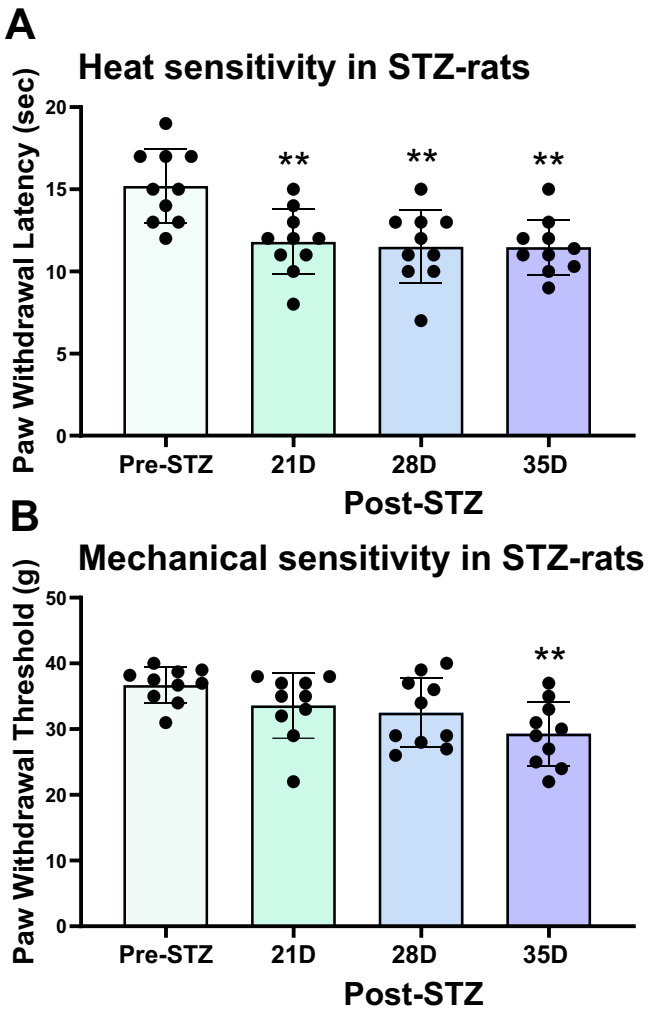

Figure 2 Behavioral signs of STZ-induced mechanical and heat hypersensitivity. Mechanical pain sentivity (**A**) and heat pain sensitivity (**B**) The data are presented as mean ± SEM. Each dot per column represents one rat. Figure 2A shows that STZ treatment caused highly significant ($p<0.01$) decreases in the mean PWL at all the time points tested (21, 28 and 35 days (**D**) post-STZ), indicating that STZ rats developed heat hypersensitivity at these time points. In contrast, there was a significant ($p<0.01$) decrease in the mean PWT only at 35 days (**D**) pos-STZ (Figure 2B), indicting a delay in the development of mechanical hypersensitivity. Note that comparisons were between pre-STZ values (before STZ treatment) and post-STZ values at different time points. Statistical tests were made with one-way ANOVA test followed with a Tukey's multiple comparison (post hoc) test. The level of statistical significance is indicated with asterisks (**$p < 0.01$).

than those we have reported previously.[15] This is probably because of the variability in pain sensitivity in different rats. It is noteworthy that both animals and humans exhibit considerable and unpredictable variability in pain sensitivity in response to the same noxious stimulus (see[25,26]). Indeed, it has been reported that the same mild noxious stimulus can elicit intense pain in one individual, yet be barely perceived by another, and that pain ratings among patients with the same condition cover the entire scale from "no pain" to "the worst pain imaginable".[26]

Flupirtine and ML213 Attenuate STZ-Induced Mechanical, but Not, Heat Hypersensitivity

Having established that STZ-rats developed behavioral signs of mechanical and heat hypersensitivity (see above), we sought to determine whether pharmacological activation of K_v7 channels with flupirtine and ML213 could reverse or attenuate these DPNP behaviors in STZ-rats. The effects of these K_v7 activators on STZ-induced mechanical and heat hypersensitivity were assessed by comparing the PWT and PWT values of post-treatment at 1–2 and 24 hours after drug treatment (post-drug) with the vehicle (negative control) and the gabapentin (positive control) values at these time points. As shown in Figure 3, compared with vehicle treatment which resulted in no significant changes in the PWT (Figure 3A) and PWL (Figure 3B) values at the

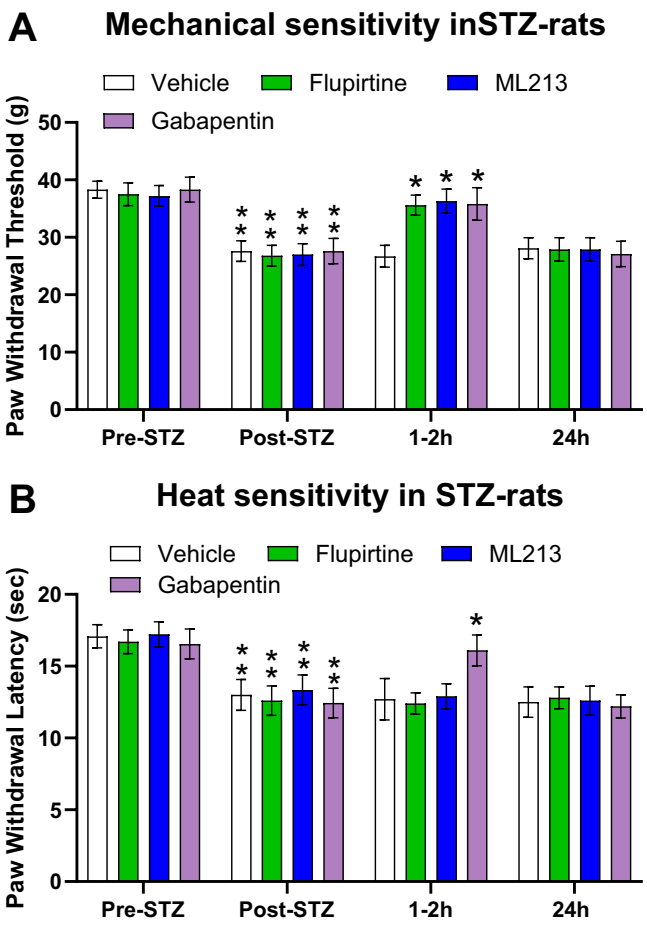

Figure 3 Effects of flupirtine, ML213 and gabapentin on STZ-induced mechanical and heat hypersensitivity. Compared to vehicle, a single injection of flupirtine at a dose of 10 mg/kg and a single injection of ML213 at a dose of 5 mg/kg significantly reversed the STZ-induced decreases in the mean PWT at 1–2 h, but not, at 24 h post-drug treatment (**A**), indicating that these K_v7 activators attenuated STZ-induced mechanical hypersensitivity. The effects of both flupirtine and ML213 were similar to those of gabapentin (10 mg/kg), which was used as the positive control. However, unlike gabapentin which also attenuated STZ-induced heat hypersensitivity (**B**), both flupirtine and ML213 given at the same doses mentioned above had no significant effect on STZ-induced decrease in PWL indicating that the heat hypersensitivity in STZ rats was not affected by these k_v7 channel agonists (**B**). Note that comparisons were between the PWT and PWL values at the two time points after drug administration (post-drug) and those of the vehicle before drug treatment 35 days after STZ. Statistical tests were made with one-way ANOVA and Tukey's multiple comparison test. The level of statistical significance is indicated with asterisks as follows: **p < 0.01; * p<0.05.

time points tested, a single injection of flupirtine (10 mg/kg) resulted in a significant increase (P<0.05) in the mean PWT at 1–2 hours post-drug treatment (Figure 3A), indicating that this prototypical K_v7 channel activator attenuated the behavioral manifestations of mechanical hypersensitivity induced by STZ. Similarly, a single injection of ML213 (5 mg/kg) caused a significant increase (P<0.05) in the mean PWT at 1–2 hours post-drug treatment (Figure 3A), indicating this ML213 also attenuated STZ-induced mechanical hypersensitivity. The anti-allodynic effects of both flupirtine and ML213 were transient and disappeared by 24-hour post-drug (Figure 3A). The short-lasting effects of flupirtine might be due to its short plasma half-life of 2.2 hours reported previously in adult rats,[27] the plasma half-life of ML213 has not been reported. Interestingly, the antiallodynic effects of flupirtine and ML213 were similar to those of the positive control gabapentin (Figure 3A) which caused significant increases ($p < 0.05$) in both the PWT (Figure 3A) and PWL (Figure 3B). In contrast, both flupirtine and ML213 caused no significant changes in PWL (Figure 2B) indicating their inability to reverse/reduce heat hypersensitivity in STZ-rats.

Blockade of K_v7 Channels with XE991 Prevents the Analgesic Effects of Flupirtine and ML213

To determine that the analgesic effects of flupirtine and ML213 are mediated by activation of K_v7 channels, we used the K_v7 channel blocker XE991 to evaluate whether the anti-allodynic effects of these K_v7 channel agonists could be

prevented by the K_v7 channel antagonist XE991. Compared with the vehicle, pretreatment (~30 min before the administration of the K_v7 channel agonists) with XE991 (3 mg/kg) significantly suppressed/prevented the increases in the PWT induced by flupirtine (15 mg/kg) and ML213 (5mg/kg) (Figure 4). These findings suggest that the anti-allodynic effects of flupirtine and ML213 were at least partially mediated by K_v7 channels. We also tested the effects of flupirtine and ML213 on PWT and PWL in naïve (normal) rats, as a control. As shown in Figure 5A and B, all of them showed no significant effect on the baseline PWT or PWL, compared with the vehicle group, indicating that these drugs had no effect on the pain sensitivity of naïve rats as we reported previously for retigabine and XE991.[15] It should be noted that the naïve rats were used to ensure that the observed anti-allodynic effects of flupirtine and ML213 are not due to their effects on the baseline pain sensitivity. The results showed that, compared with the vehicle group, neither flupirtine nor ML213 (Figure 5) had any effect on the baseline PWT or PWL, indicating that the effects of these drugs were most likely mediated by K_v7 channels. It should also be noted the values of PWT and PWL in the experimental groups (STZ rats) were not compared with those of naïve rats but they were compared with their baseline values, ie, the values before STZ injection.

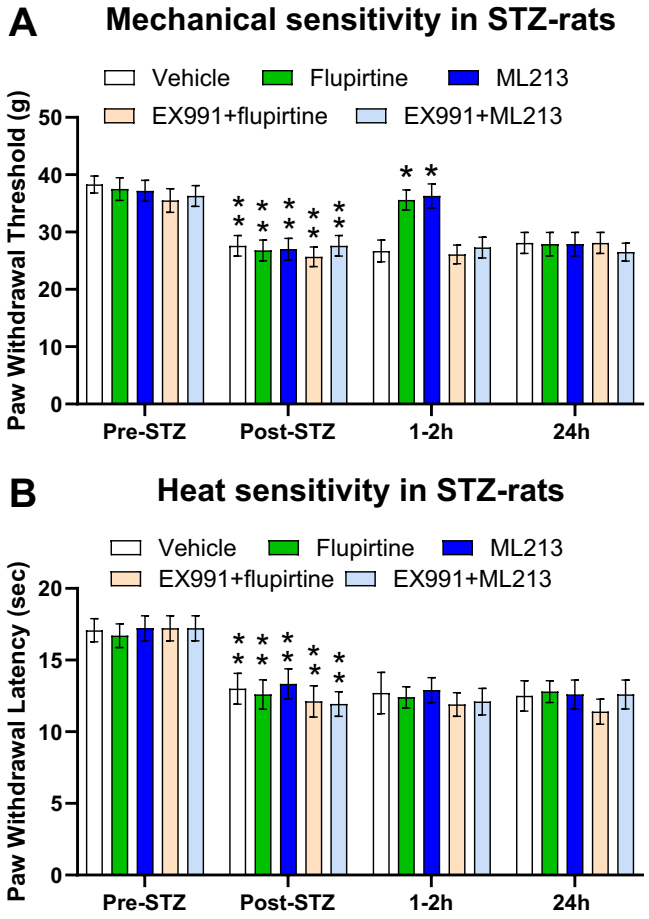

Figure 4 Effects of XE991 on the anti-allodynic effects of flupirtine and ML213 in STZ rats. STZ treatment caused significant decreases in the mean PWT (**A**) and mean PWL (**B**) 35 days post-treatment in all five groups of rats (vehicle, flupirtine, ML213, XE991 plus flupirtine and XE991 plus ML213) indicating that all rat groups developed mechanical (**A**) and heat (**B**) hypersensitivity. Compared with vehicle (n = 8 rats), single injections of flupirtine (10 mg/kg, n=8 rats) and ML213 (5 mg/kg, n=8 rats) significantly reduced mechanical hypersensitivity at 1–2 h post-drug treatment (**A**). These anti-allodynic effects of flupirtine and ML213 were prevented/blocked by pretreatment with the K_v7 channel antagonist XE991 (3mg/kg, n=8 rats) indicating that the effects of flupirtine and ML213 were mediated by Kv7 channels. Statistical tests were made with one-way repeated measures ANOVA, followed with Tukey's post-hoc test. The level of statistical significance is indicated with asterisks as follows: **$p < 0.01$; * $p<0.05$.

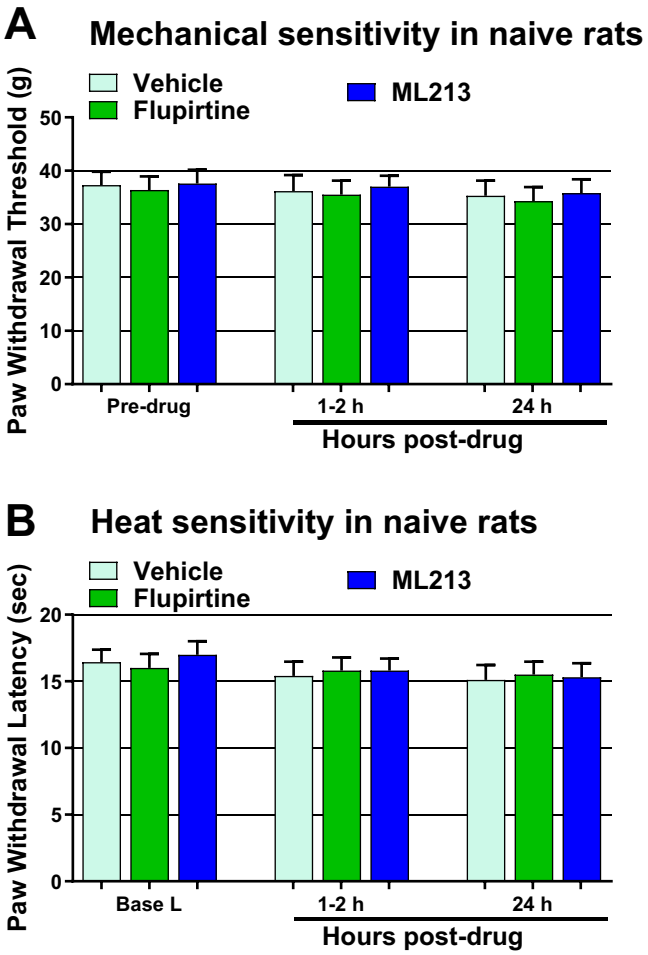

Figure 5 Effects of flupirtine and ML213 on pain sensitivity in naïve rats. Injections of flupirtine and ML213 at the same dose of 10 mg/kg and 5 mg/kg respectively into naive rats caused no significant changes in the mean PWT (**A**) or PWL (**B**) indicating they had no obvious effects on pain sensitivity on normal rats. Statistical tests were made with one–way repeated measures ANOVA.

Discussion

In the present study, we examined whether pharmacological activation of the K_v7 channels with their activators/openers flupirtine and ML213 attenuate pain hypersensitivity in the STZ rat model of DPNP. We found that: (a) STZ-diabetic rats exhibited behavioral indices of mechanical and heat hypersensitivity, (b) single injections of both flupirtine and ML213 attenuated mechanical, but not heat hypersensitivity, 35 days post-STZ, (c) the anti-allodynic effects of these K_v7 channel activators were similar to those of the gabapentin (positive control), the first-line treatment for DPNP (see,[28] and (d) their anti-allodynic effects were inhibited by the K_v7/M channel blocker XE991 indicating that their effects are mediated by K_v7 channels, and that these channels are involved in the pathogenesis of DPNP.

Preclinical studies using rodent models of DPNP suggest that, like other types of PNP, DPNP is due at least partly to abnormal hyperexcitability of DRG neurons (for review see e.g.[13,29]). This is based on several findings including that both C- and A-fiber DRG neurons exhibit aberrant SA, the key characteristic of neuronal hyperexcitability.[9–12] Reduced activity of K_v channels seems to be a hallmark of the hyperexcitability seen in many pathological settings because their activation produces membrane hyperpolarization.[30] Indeed, there is accumulating evidence that a decrease in expression and/or function of K_v channels, especially those that are tonically active near the resting membrane potential such as K_v7 channels, contributes to hyperexcitability of sensory neurons associated with nerve injury/dysfunction. K_v7 channels may contribute to such hyperexcitability because: (a) they underlie the slowly activating, non-inactivating outward M current (I_M) that normally exerts a powerful stabilizing influence on neuronal excitability (see eg,[31–34]) (b) they have been found

within both peripheral and central components of the nociceptive pathway including nociceptive peripheral nerve endings and nociceptive dorsal roots/central terminals;[35–37] for review see,[38] (c) they (K_v7.2 and/or K_v7.5 subunits) were found to be down-regulated in DRG neurons after spinal nerve injury (see[38]) and in STZ-rats,[18] (d) their inhibition with a specific blocker XE991 increases excitability of DRG neurons,[39,40] and (e) their activators/openers reduce excitability of nociceptive neurons, and block/reduce nerve injury-induced SA in sensory fibers;[41–43] (see[38] for review) and in a subpopulation of DRG neurons in STZ-rats.[12]

Anti-Allodynic Effects of Flupirtine and ML213 in STZ Rats

Activation of K_v7 channels has been proven as a useful strategy for attenuation of chronic pain in animal models (for reviews see e.g.[38,44–46]). Indeed, several animal studies have shown that activation of K_v7 channels with retigabine is effective in alleviating pain behaviors in various experimental models of pain including rodent models of trigeminal neuropathy,[47] spinal cord injury,[48] inflammatory, neuropathic and cancer pain,[39,49] (see[38] for review), chemotherapy-induced PNP[50] and DPNP.[15,51] However, the anticonvulsant retigabine, the most extensively studied compound for its actions on neuronal K_v7 channels, does not show selectivity for any particular neuronal K_v7 channel subtypes, and shows unspecific effects on other targets like GABA receptors (see[38,44] for reviews). It should be noted that in humans, retigabine was approved in 2011 by Food and Drug Administration as an adjuvant treatment of partial-onset seizures in epileptic patients, but its clinical use has been discontinued since June 2017 due to its side effects.[38,44]

A few previous animal studies have shown that the other prototypical M channel opener flupirtine (the parent compound of retigabine) also exhibits analgesic efficacy in pain models. For example, flupirtine was found to be effective in attenuating heat hypersensitivity in the partial sciatic nerve ligation model of PNP[36] and to reduce mechanical hypersensitivity in mouse models of visceral and inflammatory pain as well as chemotherapy-induced PNP.[52] Our current findings that flupirtine attenuated mechanical hypersensitivity in the STZ rat model of DPNP are in general agreement with these findings and are consistent with our previous findings that activation of K_v7 channels with retigabine dose dependently attenuated mechanical, but not heat hypersensitivity in STZ rats.[15] However, the present study is, as far as we know, the first to report that flupirtine is effective in alleviating NP behavior in the STZ model of DPNP. It is noteworthy that flupirtine was used clinically for decades in Europe as a centrally acting, nonopioid analgesic for treatment of a variety of acute and chronic pain (see[53]). However, flupirtine, like retigabine, was also reported to have unspecific effects on other targets such as NMDA and GABA receptors,[54] but little direct evidence seems to exist for this. It is thus believed that the analgesic effect of flupirtine is mainly related to its activity as a K_v7 channel opener.[55] It should be noted that flupirtine has also recently been discontinued because of its safety issues associated liver toxicity. Nevertheless, based on the findings of their clinal study,[56] Paul and co-workers concluded that flupirtine can still be used as an effective analgesic in cancer patients with chemotherapy-induced pain as long as flupirtine is given for less than two weeks to avoid drug-related hepatotoxicity.

Several other K_v7 channel modulators that are more potent and selective than retigabine have been developed (see[38,45]) including ML213 which has been shown to be much more potent than retigabine at reducing spinal reflexes[57] and to act more specifically on Kv7 channels.[58] However, its analgesic ability has not been explored until now. Indeed, the present study is the first to report that ML213 has anti-allodynic effect in the STZ model of DPNP. Interestingly, the anti-allodynic effects of flupirtine and ML213 were similar to those of the positive control gabapentin which has been reported to activate K_v7.3 and 7.5 channels when they are expressed in Xenopus oocytes.[59] Given that separate nociceptor subsets mediate mechanical and heat hypersensitivity, the lack of effects of K_v7 channel activators on heat hypersensitivity reported here, may be due to their differential effects on different nociceptor subpopulations as discussed previously.[12]

Possible Mechanisms of the Anti-Allodynic Effects of Flupirtine and ML213

It is difficult to determine precisely the mode and site of action of flupirtine and ML213, but it is possible that their anti-allodynic effects are mediated by K_v7 channels in Aβ-fiber neurons because: (a) STZ-induced mechanical allodynia in mice was reversed by selective blockade of myelinated Aβ-afferent fibers,[60] (b) capsaicin-sensitive C-fiber afferents are not required for the development of mechanical allodynia in the STZ rat model,[61] and (c) STZ-induced spontaneous activity in Aβ-fiber neurons was suppressed by retigabine.[12] These K_v7 channel activators may exert their effects by

causing membrane hyperpolarization at t-junction (the point at which the axon, stemming from the cell body, bifurcates into the peripheral and central branches) resulting in failure of an action potential propagation (see[38]) and thereby in reduction of central sensitization, which is an important mechanism of PNP.[13]

It is noteworthy that the validity of STZ-treated animals as a model of DPNP has been challenged by a number of researchers (e.g.,[62]). However, we have used/been using the STZ rat model because, it is more commonly used than other models of DPNP and because of its rapid induction, greater stability, and low cost as we discussed previously (e.g.[15]). More importantly, the STZ model has been shown by numerous animal studies to exhibit long-lasting behavioral signs of DPNP including mechanical hypersensitivity which is believed to correspond to the mechanical allodynia (cutaneous hypersensitivity) in patients who experience acute distress on contact with an external mechanical stimulus such as clothing. (see[15] and references therein).

Conclusion

The present study reports for the first time that the K_v7 channel activators flupirtine and ML213 are effective in attenuating mechanical, but not heat hypersensitivity, in STZ rats. Their anti-allodynic effects which were similar to those of gabapentin (positive control), the first-line treatment for DPNP are likely to be mediated K_v7 channels because they were prevented by the K_v7 channel blocker XE991. Taken together, the findings suggest that strategies that target activation of K_v7 channels may be effective in treating DPNP in humans.

Acknowledgments

We are grateful to the RA Dina Mohammed and staff at LARC of Qatar University for their help support.

Funding

The work is supported by a grant from Qatar Foundation (NPRP13s-0213–200352) to LD.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Abbott CA, Malik RA, van Ross ER, Kulkarni J, Boulton AJ. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. *Diabetes Care*. 2011;34(10):2220–2224. doi:10.2337/dc11-1108
2. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: Dynamic modeling of incidence, mortality, and prediabetes prevalence. *Popul Health Metr*. 2010;8:29. doi:10.1186/1478-7954-8-29
3. Feldman EL, Callaghan BC, Pop-Busui R, et al. Diabetic neuropathy. *Nat Rev Dis Primers*. 2019;5(1):42. doi:10.1038/s41572-019-0097-9
4. Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol*. 2015;14(2):162–173. doi:10.1016/S1474-4422(14)70251-0
5. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol*. 2010;9(8):807–819. doi:10.1016/S1474-4422(10)70143-5
6. Suzuki Y, Sato J, Kawanishi M, Mizumura K. Lowered response threshold and increased responsiveness to mechanical stimulation of cutaneous nociceptive fibers in streptozotocin-diabetic rat skin in vitro--correlates of mechanical allodynia and hyperalgesia observed in the early stage of diabetes. *Neurosci Res*. 2002;43(2):171–178. doi:10.1016/s0168-0102(02)00033-0
7. Garcia-Perez E, Schonberger T, Sumalla M, et al. Behavioural, morphological and electrophysiological assessment of the effects of type 2 diabetes mellitus on large and small nerve fibres in Zucker diabetic fatty, Zucker lean and Wistar rats. *Eur J Pain*. 2018;22(8):1457–1472. doi:10.1002/ejp.1235
8. Orstavik K, Namer B, Schmidt R, et al. Abnormal function of C-fibers in patients with diabetic neuropathy. *J Neurosci*. 2006;26(44):11287–11294. doi:10.1523/JNEUROSCI.2659-06.2006
9. Burchiel KJ, Russell LC, Lee RP, Sima AA. Spontaneous activity of primary afferent neurons in diabetic BB/Wistar rats. A possible mechanism of chronic diabetic neuropathic pain. *Diabetes*. 1985;34(11):1210–1213. doi:10.2337/diab.34.11.1210
10. Ahlgren SC, Wang JF, Levine JD. C-fiber mechanical stimulus-response functions are different in inflammatory versus neuropathic hyperalgesia in the rat. *Neuroscience*. 1997;76(1):285–290. doi:10.1016/s0306-4522(96)00290-4
11. Chen X, Levine JD. Hyper-responsivity in a subset of C-fiber nociceptors in a model of painful diabetic neuropathy in the rat. *Neuroscience*. 2001;102(1):185–192. doi:10.1016/s0306-4522(00)00454-1
12. Djouhri L, Zeidan A, El-Aleem SA A, Smith T. Cutaneous abeta-non-nociceptive, but not c-nociceptive, dorsal root ganglion neurons exhibit spontaneous activity in the streptozotocin rat model of painful diabetic neuropathy in vivo. *Front Neurosci*. 2020;14:530. doi:10.3389/fnins.2020.00530
13. Campbell JN, Meyer RA. Mechanisms of neuropathic pain. *Neuron*. 2006;52(1):77–92. doi:10.1016/j.neuron.2006.09.021

14. Brown DA, Passmore GM. Neural KCNQ (Kv7) channels. *Br J Pharmacol Apr*. 2009;156(8):1185–1195. doi:10.1111/j.1476-5381.2009.00111.x
15. Djouhri L, Malki MI, Zeidan A, Nagi K, Smith T. Activation of Kv7 channels with the anticonvulsant retigabine alleviates neuropathic pain behaviour in the streptozotocin rat model of diabetic neuropathy. *J Drug Target*. 2019;27(10):1118–1126. doi:10.1080/1061186X.2019.1608552
16. Rundfeldt C, Netzer R. Investigations into the mechanism of action of the new anticonvulsant retigabine. Interaction with GABAergic and glutamatergic neurotransmission and with voltage gated ion channels. *Arzneimittelforschung*. 2000;50(12):1063–1070. doi:10.1055/s-0031-1300346
17. Jolivalt CG, Frizzi KE, Guernsey L, et al. Peripheral Neuropathy in Mouse Models of Diabetes. *Curr Protoc Mouse Biol*. 2016;6(3):223–255. doi:10.1002/cpmo.11
18. Djouhri L, Zeidan A, Abd El-Aleem SA. Changes in expression of Kv7.5 and Kv7.2 channels in dorsal root ganglion neurons in the streptozotocin rat model of painful diabetic neuropathy. *Neurosci Lett*. 2020;736:135277. doi:10.1016/j.neulet.2020.135277
19. Zhang F, Liu S, Jin L, et al. Antinociceptive Efficacy of Retigabine and Flupirtine for Gout Arthritis Pain. *Pharmacology*. 2020;105(7–8):471–476. doi:10.1159/000505934
20. McGuier NS, Rinker JA, Cannady R, et al. Identification and validation of midbrain Kcnq4 regulation of heavy alcohol consumption in rodents. *Neuropharmacology*. 2018;138:10–19. doi:10.1016/j.neuropharm.2018.05.020
21. Amato G, Roeloffs R, Rigdon GC, et al. N-Pyridyl and Pyrimidine Benzamides as KCNQ2/Q3 Potassium Channel Openers for the Treatment of Epilepsy. *ACS Med Chem Lett*. 2011;2(6):481–484. doi:10.1021/ml200053x
22. Yu H, Wu M, Townsend SD, et al. Discovery, synthesis, and structure activity relationship of a series of N-Aryl- bicyclo[2.2.1]heptane-2-carboxamides: characterization of ML213 as a novel KCNQ2 and KCNQ4 potassium channel opener. *ACS Chem Neurosci*. 2011;2(10):572–577. doi:10.1021/cn200065b
23. Kim RY, Yau MC, Galpin JD, et al. Atomic basis for therapeutic activation of neuronal potassium channels. *Nat Commun*. 2015;6:8116. doi:10.1038/ncomms9116
24. Provence A, Angoli D, Petkov GV. K(V)7 channel pharmacological activation by the novel activator ML213: Role for heteromeric K(V)7.4/K(V)7.5 channels in guinea pig detrusor smooth muscle function. *J Pharmacol Exp Ther*. 2018;364(1):131–144. doi:10.1124/jpet.117.243162
25. Nielsen CS, Staud R, Price DD. Individual differences in pain sensitivity: measurement, causation, and consequences. *J Pain*. 2009;10(3):231–237. doi:10.1016/j.jpain.2008.09.010
26. Mogil JS. Sources of Individual Differences in Pain. *Annu Rev Neurosci*. 2021;44:1–25. doi:10.1146/annurev-neuro-092820-105941
27. Obermeier K, Niebch G, Thiemer K. Pharmacokinetics and biotransformation of the analgesic flupirtine in the rat and dog. *Arzneimittelforschung*. 1985;35(1):60–67.
28. Davari M, Amani B, Amani B, Khanijahani A, Akbarzadeh A, Shabestan R. Pregabalin and gabapentin in neuropathic pain management after spinal cord injury: a systematic review and meta-analysis. *Korean J Pain*. 2020;33(1):3–12. doi:10.3344/kjp.2020.33.1.3
29. Feldman EL, Nave KA, Jensen TS, Bennett DLH. New horizons in diabetic neuropathy: Mechanisms, bioenergetics, and pain. *Neuron*. 2017;93(6):1296–1313. doi:10.1016/j.neuron.2017.02.005
30. Tsantoulas C. Emerging potassium channel targets for the treatment of pain. *Curr Opin Support Palliat Care*. 2015;9(2):147–154. doi:10.1097/SPC.0000000000000131
31. Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci*. 2000;1(1):21–30. doi:10.1038/35036198
32. Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat Rev Neurosci*. 2005;6(11):850–862. doi:10.1038/nrn1785
33. Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. *Pharmacol Ther Apr*. 2001;90(1):1–19. doi:10.1016/s0163-7258(01)00116-4
34. Rivera-Arconada I, Roza C, Lopez-Garcia JA. Enhancing m currents: a way out for neuropathic pain? *Front Mol Neurosci*. 2009;2:10. doi:10.3389/neuro.02.010.2009
35. Rivera-Arconada I, Lopez-Garcia JA. Retigabine-induced population primary afferent hyperpolarisation in vitro. *Neuropharmacology*. 2006;51(4):756–763. doi:10.1016/j.neuropharm.2006.05.015
36. Rose K, Ooi L, Dalle C, Robertson B, Wood IC, Gamper N. Transcriptional repression of the M channel subunit Kv7.2 in chronic nerve injury. *Pain*. 2011;152(4):742–754. doi:10.1016/j.pain.2010.12.028
37. Passmore GM, Reilly JM, Thakur M, et al. Functional significance of M-type potassium channels in nociceptive cutaneous sensory endings. *Front Mol Neurosci*. 2012;5:63. doi:10.3389/fnmol.2012.00063
38. Du X, Gao H, Jaffe D, Zhang H, Gamper N. M-type K(+) channels in peripheral nociceptive pathways. *Br J Pharmacol*. 2018;175(12):2158–2172. doi:10.1111/bph.13978
39. Passmore GM, Selyanko AA, Mistry M, et al. KCNQ/M currents in sensory neurons: significance for pain therapy. *J Neurosci*. 2003;23(18):7227–7236. doi:10.1523/JNEUROSCI.23-18-07227.2003
40. Gamper N, Zaika O, Li Y, et al. Oxidative modification of M-type K(+) channels as a mechanism of cytoprotective neuronal silencing. *EMBO J*. 2006;25(20):4996–5004. doi:10.1038/sj.emboj.7601374
41. Lang PM, Fleckenstein J, Passmore GM, Brown DA, Grafe P. Retigabine reduces the excitability of unmyelinated peripheral human axons. *Neuropharmacology*. 2008;54(8):1271–1278. doi:10.1016/j.neuropharm.2008.04.006
42. Roza C, Lopez-Garcia JA. Retigabine, the specific KCNQ channel opener, blocks ectopic discharges in axotomized sensory fibres. *Pain*. 2008;138(3):537–545. doi:10.1016/j.pain.2008.01.031
43. Bernal L, Lopez-Garcia JA, Roza C. Spontaneous activity in C-fibres after partial damage to the saphenous nerve in mice: effects of retigabine. *Eur J Pain*. 2016;20(8):1335–1345. doi:10.1002/ejp.858
44. Abd-Elsayed A, Jackson M, Gu SL, Fiala K, Gu J. Neuropathic pain and Kv7 voltage-gated potassium channels: the potential role of Kv7 activators in the treatment of neuropathic pain. *Mol Pain*. 2019;15:1744806919864256. doi:10.1177/1744806919864256
45. Alles SRA, Smith PA. Peripheral voltage-gated cation channels in neuropathic pain and their potential as therapeutic targets. *Front Pain Res*. 2021;2:750583. doi:10.3389/fpain.2021.750583
46. Busserolles J, Tsantoulas C, Eschalier A, Lopez Garcia JA. Potassium channels in neuropathic pain: advances, challenges, and emerging ideas. *Pain*. 2016;157(Suppl 1):S7–S14. doi:10.1097/j.pain.0000000000000368

47. Abd-Elsayed AA, Ikeda R, Jia Z, et al. KCNQ channels in nociceptive cold-sensing trigeminal ganglion neurons as therapeutic targets for treating orofacial cold hyperalgesia. *Mol Pain*. 2015;11:45. doi:10.1186/s12990-015-0048-8
48. Wu Z, Li L, Xie F, et al. Activation of KCNQ channels suppresses spontaneous activity in dorsal root ganglion neurons and reduces chronic pain after spinal Cord Injury. *J Neurotrauma*. 2017;34(6):1260–1270. doi:10.1089/neu.2016.4789
49. Blackburn-Munro G, Jensen BS. The anticonvulsant retigabine attenuates nociceptive behaviours in rat models of persistent and neuropathic pain. *Eur J Pharmacol*. 2003;460(2–3):109–116. doi:10.1016/s0014-2999(02)02924-2
50. Ling J, Erol F, Viatchenko-Karpinski V, Kanda H, Gu JG. Orofacial neuropathic pain induced by oxaliplatin: downregulation of KCNQ2 channels in V2 trigeminal ganglion neurons and treatment by the KCNQ2 channel potentiator retigabine. *Mol Pain*. 2017;13:1744806917724715. doi:10.1177/1744806917724715
51. Yu T, Li L, Liu H, Li H, Liu Z, Li Z. KCNQ2/3/5 channels in dorsal root ganglion neurons can be therapeutic targets of neuropathic pain in diabetic rats. *Mol Pain*. 2018;14:1744806918793229. doi:10.1177/1744806918793229
52. Chen Y, Xiao X, Huang C, et al. Flupirtine and antihistamines exert synergistic anti-nociceptive effects in mice. *Psychopharm*. 2023;240 (4):881–897. doi:10.1007/s00213-023-06329-3
53. Szelenyi I. Flupirtine, a re-discovered drug, revisited. *Inflamm Res Mar*. 2013;62(3):251–258. doi:10.1007/s00011-013-0592-5
54. Miceli F, Soldovieri MV, Martire M, Taglialatela M. Molecular pharmacology and therapeutic potential of neuronal Kv7-modulating drugs. *Curr Opin Pharmacol*. 2008;8(1):65–74. doi:10.1016/j.coph.2007.10.003
55. Devulder J. Flupirtine in pain management: pharmacological properties and clinical use. *CNS Drugs*. 2010;24(10):867–881. doi:10.2165/11536230-000000000-00000
56. Paul A, Razak MA, Binoy A, Devi PU, Pavithran K. Flupirtine in the Management of Taxane-induced Pain in Cancer Patients. *Indian J Palliat Care*. 2022;28(3):262–265. doi:10.25259/IJPC_41_2021
57. Vicente-Baz J, Lopez-Garcia JA, Rivera-Arconada I. Effects of novel subtype selective M-current activators on spinal reflexes in vitro: comparison with retigabine. *Neuropharmacology*. 2016;109:131–138. doi:10.1016/j.neuropharm.2016.05.025
58. Rivera-Arconada I, Vicente-Baz J, Lopez-Garcia JA. Targeting Kv7 channels in pain pathways. *Oncotarget*. 2017;8(8):12554–12555. doi:10.18632/oncotarget.15261
59. Manville RW, Abbott GW. Ancient and modern anticonvulsants act synergistically in a KCNQ potassium channel binding pocket. *Nat Commun*. 2018;9(1):3845. doi:10.1038/s41467-018-06339-2
60. Xu ZZ, Kim YH, Bang S, et al. Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. *Nat Med*. 2015;21 (11):1326–1331. doi:10.1038/nm.3978
61. Khan GM, Chen SR, Pan HL. Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats. *Neuroscience*. 2002;114 (2):291–299. doi:10.1016/s0306-4522(02)00372-x
62. Bishnoi M, Bosgraaf CA, Abooj M, Zhong L, Premkumar LS. Streptozotocin-induced early thermal hyperalgesia is independent of glycemic state of rats: role of transient receptor potential vanilloid 1(TRPV1) and inflammatory mediators. *Mol Pain*. 2011;7:52. doi:10.1186/1744-8069-7-52