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Original Research

Alleviation of paclitaxel-induced mechanical hypersensitivity and hyperalgesic priming with AMPK activators in male and female mice

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ABSTRACT

AMP-activated protein kinase (AMPK) is an energy-sensing kinase that has emerged as a novel therapeutic target for pain due to its ability to inhibit mechanistic target of rapamycin (mTOR) and mitogen activated protein kinase (MAPK) signaling, two signaling pathways that are linked to pain promotion after injury as well as the development of hyperalgesic priming. MAPK and mTOR signaling are also implicated in chemotherapy induced peripheral neuropathy (CIPN). We conducted a series of experiments to gain further insight into how AMPK activators might best be used to treat pain in both sexes in the setting of CIPN from paclitaxel. We also assessed whether hyperalgesic priming emerges from paclitaxel treatment and if this can be prevented by AMPK targeting. AMPK can be pharmacologically activated indirectly through regulation of upstream kinases like liver kinase B1 (LKB1) or directly using positive allosteric modulators. We used the indirect AMPK activators metformin and narciclasine, both of which have been shown to reduce pain in preclinical models but with much different potencies and different efficacies depending on the sex of the animal. We used the direct AMPK activator MK8722 because it is the most potent and specific such activator described to date. Here, the AMPK activators were used in 2 different treatment paradigms. First the drugs were given concurrently with paclitaxel to test whether they prevent mechanical hypersensitivity. Second the AMPK activators were given after the completion of paclitaxel treatment to test whether they reverse established mechanical hypersensitivity. Consistent with our previously published findings with metformin, narciclasine (1 mg/kg) produced an anti-hyperalgesic effect, preventing paclitaxel-induced neuropathy in outbred mice of both sexes. In contrast to metformin, narciclasine also reversed mechanical hypersensitivity in established CIPN. Both metformin (200 mg/kg) and narciclasine prevented the development of hyperalgesic priming induced by paclitaxel treatment. MK8722 (30 mg/kg) had no effect on mechanical hypersensitivity caused by paclitaxel in either the prevention or reversal treatment paradigms. However, MK8722 did attenuate hyperalgesic priming in male and female mice. We conclude that paclitaxel induces robust hyperalgesic priming that is prevented by AMPK targeting and that narciclasine is a particularly attractive candidate for further development as a CIPN treatment.

1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is characterized by a glove-and-stocking (Seretny et al., 2014; Ma et al., 2018) distribution of pain and develops in 30–40% of cancer patients receiving chemotherapy (Pachman et al., 2011). In these patients, the pain, numbness and tingling in hands and feet develops during treatment and frequently continues even after completion of therapy. This pervasive peripheral neuropathy is a major dose-limiting adverse effect of multiple chemotherapeutic agents, particularly paclitaxel which causes CIPN in a high proportion of patients (Colvin, 2019). Currently, there are no effective treatments for chemotherapy-induced neuropathy (Brown et al., 2019). Therefore, identification of novel or existing drugs that are effective in preventing or reversing CIPN would greatly benefit treatment and quality of life of cancer patients undergoing chemotherapy treatment.

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Metformin, a well-known anti-diabetic drug, activates adenosine monophosphate-activated protein kinase (AMPK) indirectly in a liver kinase B1 (LKB1) dependent fashion (Shaw et al., 2005). AMPK detects changes in the AMP/ATP ratio to regulate anabolic processes when cellular energy status is low (Kahn et al., 2005). Several studies have shown that AMPK plays a key role in nociceptive sensitization (Song et al., 2015; Bullón et al., 2016). Recent findings show that metformin reverses established mechanical allodynia in models of neuropathic pain induced by spinal nerve ligation in rats (Melemedjian et al., 2013) and spared nerve injury in mice (Melemedjian and Khoutorsky, 2015; Inyang et al., 2019a), but these positive effects are only seen in male animals (Burton et al., 2017). On the other hand, metformin prevents CIPN in female mice but only when given concurrently with the chemotherapeutic treatment (Mao-Ying et al., 2014). Furthermore, metformin prevents hyperalgesic priming induced by plantar incision, a model of the transition from acute to chronic pain (Reichling and Levine, 2009; Price and Inyang, 2015), but again, only in male mice (Inyang et al., 2019b). Finally, several recent clinical trials suggest that metformin can be effective for mitigation of pain in humans (Taylor et al., 2013; Kialka et al., 2016; El-Fatatry et al., 2018), supporting the clinical translational potential of these preclinical findings. A major unresolved issue is whether the sex-specific effects of metformin are observed across models. Because metformin can prevent CIPN development in female mice (Mao-Ying et al., 2014), this is an ideal model system to assess the effects of this drug on pain more thoroughly. Another question is whether paclitaxel treatment can produce hyperalgesic priming. The presence of hyperalgesic priming caused by paclitaxel would suggest a long-lasting impact of chemotherapy treatment on the nervous system. Understanding mechanisms causing these changes could reveal new therapeutic options for this important type of neuropathic pain.

Various other AMPK activators have emerged in recent years that are more potent and efficacious than metformin (Cao et al., 2018; Qin et al., 2018). In experiments done to date, none of these other AMPK activators show sex-specific effects on pain outcomes in preclinical models (Inyang et al., 2019b). A particularly potent and efficacious AMPK activator for therapeutic development is narciclasine. It is a natural compound from the plant *Amaryllidaceae* (Dumont et al., 2007) that indirectly activates AMPK (Julien et al., 2017a,b) and strongly inhibits incision-evoked mechanical hypersensitivity and hyperalgesic priming in male and female mice (Inyang et al., 2019b). While indirect AMPK activators stimulate upstream kinases that phosphorylate AMPK, another way to activate AMPK is via direct, positive allosteric mechanisms. A highly selective and potent positive allosteric modulator of AMPK called MK8722 was recently described (Feng et al., 2018; Weihrauch and Handschin, 2018). We have recently compared direct (MK8722) and indirect (metformin and narciclasine) AMPK activators in an incisional pain model. Our findings demonstrate that narciclasine produces more efficacious relief of mechanical hypersensitivity than MK8722 (Inyang et al., 2019b). This likely occurs because the indirect AMPK activator narciclasine is capable of overcoming the suppression of AMPK phosphorylation that is induced by injury (Julien et al., 2017a,b; Inyang et al., 2019b). Interestingly, while indirect AMPK activators reduce acute mechanical hypersensitivity and attenuate hyperalgesic priming caused by hindpaw incision, direct AMPK activators only attenuate hyperalgesic priming in this model (Inyang et al., 2019b).

Based on the findings described above, we hypothesized that narciclasine would efficaciously prevent mechanical hypersensitivity caused by paclitaxel treatment and that it would attenuate hyperalgesic priming in male and female mice. We further hypothesized that MK8722 would have little effect on prevention of paclitaxel-induced mechanical hypersensitivity but that it would attenuate hyperalgesic priming in both sexes. Finally, we hypothesized that metformin would show similar effects to narciclasine in both male and female mice. The experiments described below were designed to test these hypotheses.

2. Methods

2.1. Laboratory animals

Animal procedures were approved by The University of Texas at Dallas Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health Guidelines. All the experiments were performed on male and female ICR outbred mice obtained from Envigo at 4 weeks of age or bred in a colony at University of Texas at Dallas. Mice were housed in the University of Texas at Dallas Animal Care Facility for at least one week prior to the start of behavioral testing. Animals had *ad libitum* access to food and water and were on a 12 hr non-inverted light/dark cycle. Experimenters were blinded to treatment groups in behavioral experiments. Mice were randomized to treatment groups using a random number generator and in such a manner that multiple treatment groups were always found within any individual cage of animals. Male and female mice were housed separately in groups of 4 per cage.

2.2. Behavioral testing and drug administration

2.2.1. Paclitaxel treatment

CIPN was induced using paclitaxel (Sigma Aldrich, 4 mg/kg) given via intraperitoneal injection (i.p.) every other day for one week for a cumulative dose of 16 mg/kg (Megat et al., 2019). Paclitaxel was dissolved in a 50/50 Kolliphor EL (Sigma Aldrich)/ ethanol solution then further dissolved in 0.9% saline for injections. Mechanical sensitivity was assessed using stimulation of the left hindpaw of the mouse with calibrated von Frey filaments from Stoelting. We used 0.6, 1.0 and 1.4-gram filaments and measured the response frequency to 10 consecutive stimulations of the hindpaw with each filament with stimulations spaced by at least 5 sec following 45 min of habituation to the testing boxes. The number of responses for each filament force was recorded with the tester blinded to the treatment groups. Withdrawal frequencies are presented as the percentage of times responding for each individual filament. Two different treatment paradigms were used in this study. In the prevention paradigm, paclitaxel and an AMPK activator were given concurrently for 1 week. In the reversal paradigm, paclitaxel was given for a week followed by 1 week of AMPK activator treatment. Mice were tested for mechanical hypersensitivity periodically until response frequency returned to baseline levels.

2.2.2. Hyperalgesic priming

Following the return to baseline, hyperalgesic priming was tested by giving each animal an intraplantar injection of prostaglandin E_2 (PGE_2) (Cayman Chemical, 100 ng/25 μL) into the left hindpaw. Response frequency following PGE_2 administration was tested at time points indicated in graphs. The PGE_2 priming experiments were modeled after previous priming experiments done in the plantar incision model with and without AMPK activator treatment (Tillu et al., 2012; Burton et al., 2017; Inyang et al., 2019b). The different AMPK activators used in this experiment were metformin i.p. (LKT Laboratories, 200 mg/kg), narciclasine by oral gavage (p.o., Santa Cruz Biotech, 1 mg/kg) (Julien et al., 2017a,b) and MK-8722p.o. (gift from Merck, 30 mg/kg) (Feng et al., 2018). Metformin was dissolved in 0.9% saline and narciclasine was made in 45% w/v (2-Hydroxypropyl)-β-cyclodextrin (Sigma Aldrich). MK-8722 was administered in 0.25% methyl cellulose (MC), 5% Tween-80, and 0.02% sodium dodecyl sulfate (SDS) (Feng et al., 2018).

2.2.3. Statistics

Data are shown as mean ± standard error of the mean (SEM) and the number of animals or samples used in each analysis are given in figure legends. GraphPad Prism 8 was used to analyze data for statistical tests, which are given in figure legends. Repeated measures two-way ANOVAs were used to analyze von Frey data. Post-hoc tests used were the Bonferroni multiple comparisons test. Significance level was

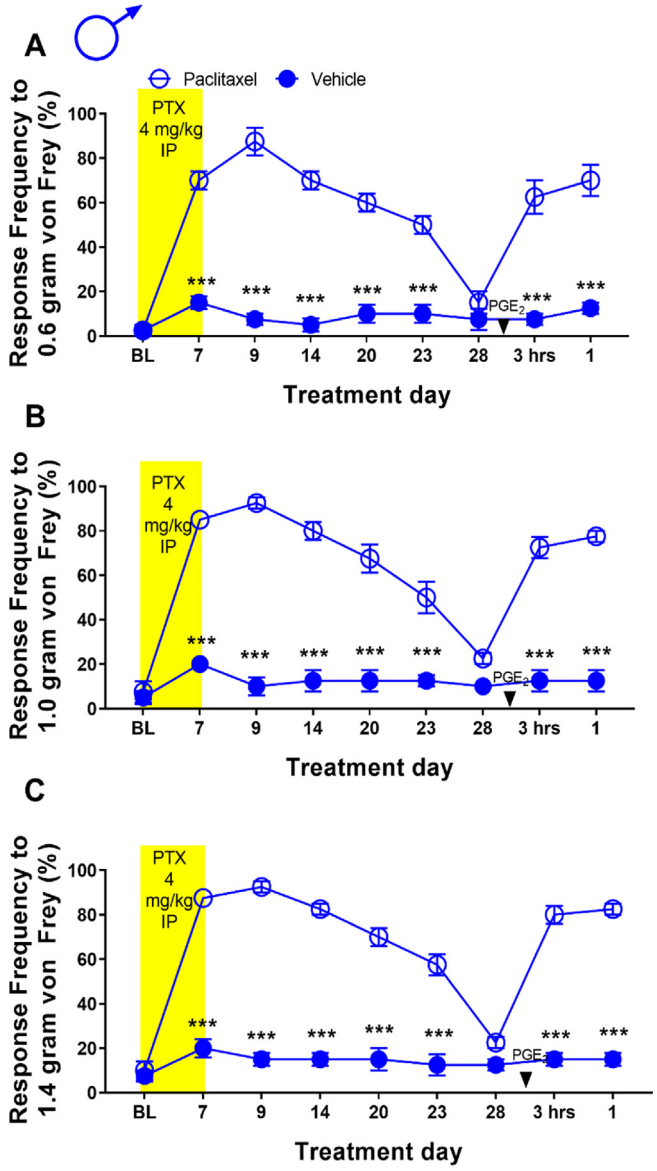

Fig. 1. Paclitaxel induced hyperalgesic priming in male mice. A-C. Paclitaxel treatment caused an increase in mechanical hypersensitivity. Following resolution of this hypersensitivity, PGE_2 was able to cause an increase in response frequency indicating the presence of hyperalgesic priming. ***p < 0.001; N = 4 per group.

set at $\alpha < 0.05$. Values for all test statistics are given in Supplementary Table 1.

3. Results

3.1. Paclitaxel induced hyperalgesic priming in male mice

Our previous work in the hindpaw incision model indicates that indirect AMPK activators reduce acute mechanical hypersensitivity and attenuate hyperalgesic priming whereas direct AMPK activators only attenuate hyperalgesic priming (Inyang et al., 2019b). We hypothesized that similar effects might be seen in a CIPN model, however, no published studies have assessed whether hyperalgesic priming is produced by treatment with chemotherapeutics like paclitaxel. To test whether paclitaxel can cause hyperalgesic priming, we first obtained baseline response frequency measures. Then mice received i.p. paclitaxel or vehicle treatment. Once the mice returned to baseline level mechanical sensitivity, animals received 100 ng PGE_2 into the left hindpaw. Only the mice that received paclitaxel demonstrated mechanical hypersensitivity at the time points tested indicating that paclitaxel causes robust hyperalgesic priming (Fig. 1A-C).

3.2. Metformin effects on paclitaxel-induced mechanical hypersensitivity and hyperalgesic priming in male and female mice

Previous studies have demonstrated that metformin treatment during the course of chemotherapy administration prevents the development of CIPN in female mice. However, if treatment is given after CIPN is established, it fails to reverse the behavioral signs of neuropathy (Mao-Ying et al., 2014). Other studies in the spared nerve injury and incisional models in mice have demonstrated that metformin reverses and prevents, respectively, mechanical hypersensitivity, but only in males (Inyang et al., 2019b; Inyang et al., 2019a). Hence, there is a discrepancy in the efficacy of metformin in male and female mice depending on the model and time point of intervention. Having shown that hyperalgesic priming is present in the paclitaxel CIPN model, we reasoned that metformin might be effective in reversing paclitaxel-induced hyperalgesic priming in both male and female mice.

To test the effects of metformin on paclitaxel-induced mechanical hypersensitivity in male mice, animals were given paclitaxel. These mice were then allocated into metformin (200 mg/kg) and vehicle treatment groups. Consistent with previous results (Mao-Ying et al., 2014), metformin did not reverse established paclitaxel-induced mechanical hypersensitivity in males. However, metformin did attenuate hyperalgesic priming (Fig. 2A-C). To test whether similar effects would be observed in female mice paclitaxel treatment was given over the course of a week followed by a week of metformin treatment. Like with male mice, metformin had no effect on the mechanical hypersensitivity caused by paclitaxel but did attenuate hyperalgesic priming (Fig. 2D-F). Therefore, unlike the hindpaw incision model where effects on hyperalgesic priming were only observed in male mice (Inyang et al., 2019b), metformin is effective in reversing paclitaxel-induced hyperalgesic priming in both sexes.

3.3. Narciclasine effects on paclitaxel-induced mechanical hypersensitivity and hyperalgesic priming in both sexes

We then tested the effects of a structurally distinct indirect AMPK activator, narciclasine. Our previous studies in the hindpaw incision model demonstrate that narciclasine dose-dependently inhibits mechanical hypersensitivity and hyperalgesic priming induced by incision in both sexes (Inyang et al., 2019b). In male mice, under the prevention treatment paradigm, narciclasine both prevented mechanical hypersensitivity and attenuated development of hyperalgesic priming following paclitaxel (Fig. 3A-C). Just as in male mice, narciclasine prevented paclitaxel-induced mechanical hypersensitivity in female mice and attenuated paclitaxel-induced priming (Fig. 3D-F). In both sexes, the effect of narciclasine on mechanical hypersensitivity persisted after cessation of treatment.

We then tested narciclasine's ability to reverse paclitaxel-induced mechanical hypersensitivity. Unlike with metformin, narciclasine was able to significantly inhibit mechanical hypersensitivity from paclitaxel in addition to attenuating hyperalgesic priming in males (Fig. 4A-C). In female mice, narciclasine also inhibited paclitaxel-induced mechanical hypersensitivity in the reversal treatment paradigm in addition to reducing hyperalgesic priming (Fig. 4D-F). In these experiments, where narciclasine was given after paclitaxel treatment, we also observed a continued effect of the drug even after cessation of treatment. Comparing the prevention to the reversal treatment paradigms, narciclasine was clearly more effective in the prevention paradigm where it almost completely attenuated development of mechanical hypersensitivity in both sexes. In the reversal paradigm, the effect of drug was significant, but there was not a complete reversal of mechanical hypersensitivity or

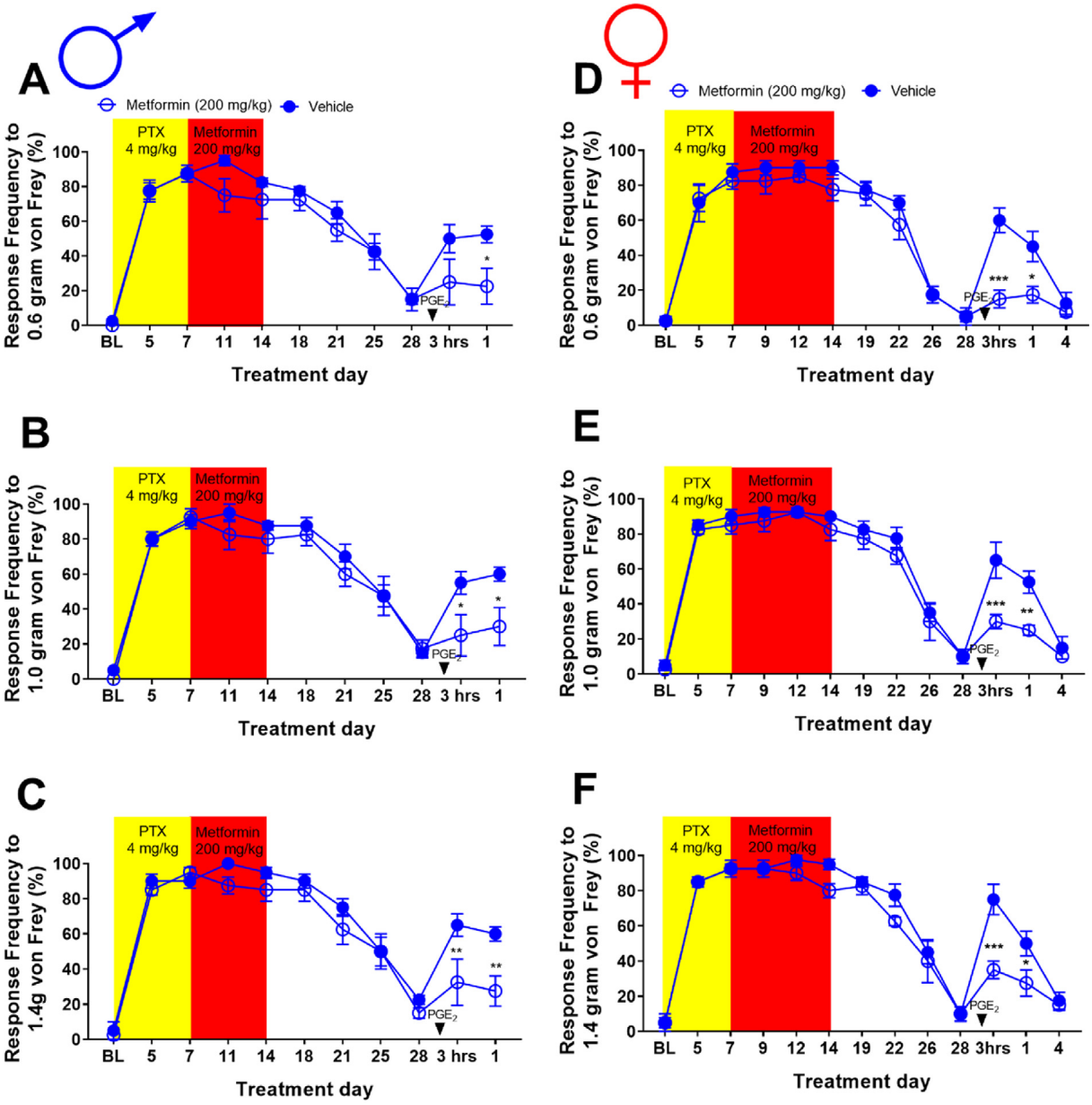

Fig. 2. Metformin attenuated paclitaxel-induced hyperalgesic priming in male and female mice. A-F. In male and female mice, metformin treatment immediately following a 7-day paclitaxel treatment failed to reverse CIPN but attenuated subsequent hyperalgesic priming precipitated by PGE_2. *p < 0.05, **p < 0.01, ***p < 0.001; N = 4 per group.

hyperalgesic priming.

3.4. MK8722 effects on paclitaxel-induced mechanical hypersensitivity and hyperalgesic priming in male and female mice

Metformin and narciclasine activate AMPK indirectly via upstream signaling mechanisms. MK8722 is a structurally distinct AMPK activator that binds directly to the kinase to allosterically increase kinase activity. We tested this direct AMPK activator in males and females in paclitaxel CIPN prevention and reversal paradigms. MK8722 did not prevent mechanical hypersensitivity in male mice but did reduce hyperalgesic priming (Fig. 5A-C). MK8722 also did not prevent paclitaxel-induced mechanical hypersensitivity in female mice when given concurrently with paclitaxel but it did attenuate paclitaxel-induced hyperalgesic priming (Fig. 5D-F).

With another set of male mice, we assessed the ability of MK8722 to reverse paclitaxel-induced neuropathy. These mice received paclitaxel for a week followed by a week of MK8722 treatment. MK8722 was not able to reverse mechanical hypersensitivity induced by paclitaxel but it again attenuated hyperalgesic priming (Fig. 6A-C). In females, MK8722 also did not reverse established mechanical hypersensitivity but did attenuate hyperalgesic priming (Fig. 6D-F).

4. Discussion

One of the key findings of this study is that paclitaxel can induce hyperalgesic priming and that this priming effect is strongly attenuated by AMPK activator treatment. All of the direct and indirect AMPK activators used successfully reduced and/or reversed paclitaxel-induced hyperalgesic priming, even though narciclasine was the only one capable of both preventing and reversing paclitaxel-induced mechanical hypersensitivity. Hyperalgesic priming effects have not been previously explored in models of CIPN, but our work makes it clear that paclitaxel treatment produces a robust hyperalgesic priming effect that is

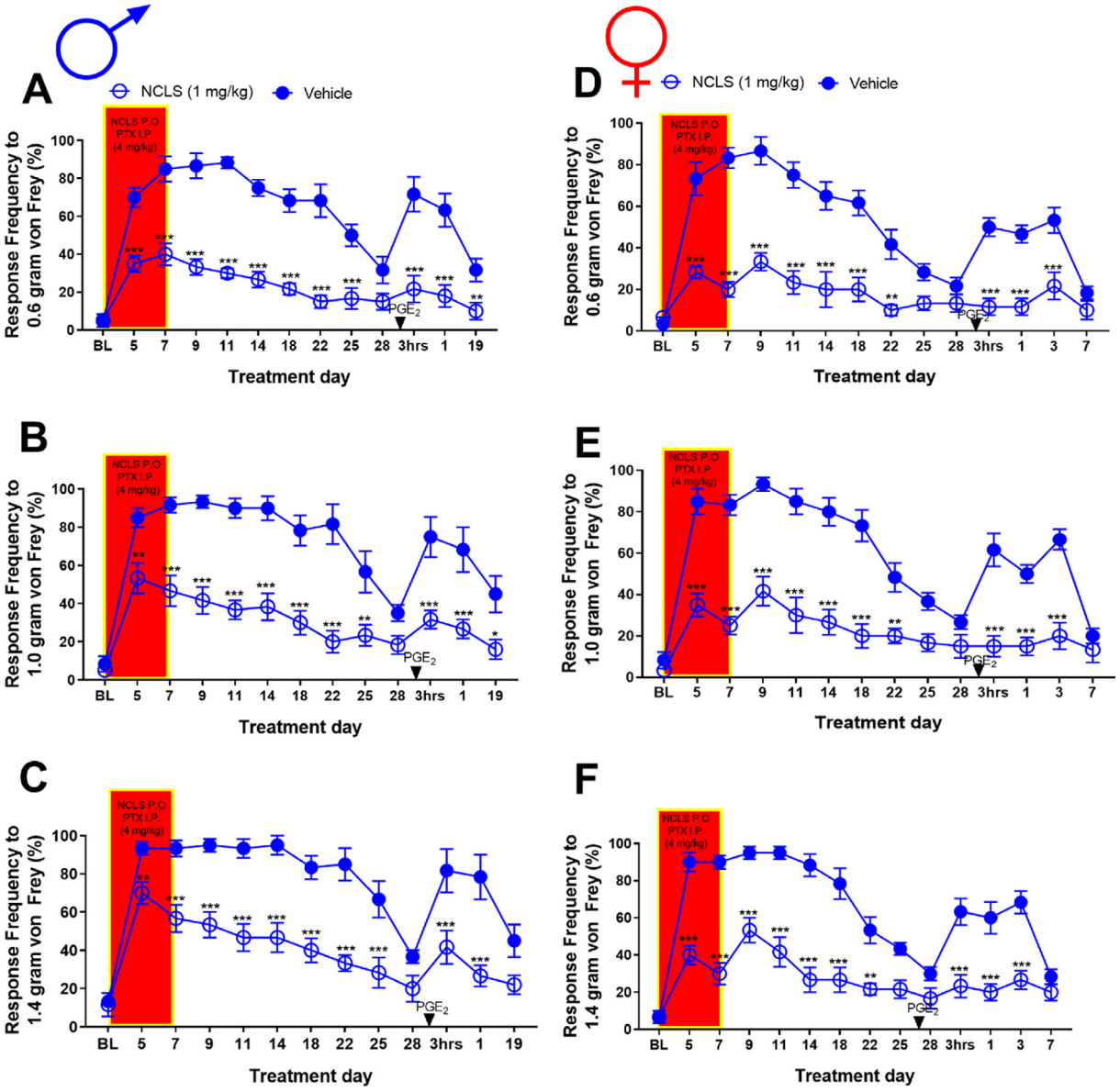

Fig. 3. Narciclasine (NCLS) attenuated paclitaxel-induced mechanical hypersensitivity and hyperalgesic priming in males and females. A-C. NCLS attenuated the development of CIPN in male mice when give concurrently with paclitaxel and reduced hyperalgesic priming precipitated by PGE_2. *p < 0.05; **p < 0.01; ***p < 0.001; N = 6 for NCLS group and vehicle group. D-F. NCLS prevented CIPN in female mice when given concurrently with paclitaxel treatment and attenuated hyperalgesic priming precipitated by PGE_2. *p < 0.05; **p < 0.01; ***p < 0.001; N = 6 for NCLS group and N = 6 for vehicle group.

apparent in both male and female mice. Consistent with our previous work in the incisional model, narciclasine, metformin and MK8722 were all capable of inhibiting hyperalgesic priming revealed by mechanical hypersensitivity in response to PGE_2 injection. These findings demonstrate that paclitaxel treatment creates an enduring change in the nervous system that enhances the response to subsequent challenge to a normally non-noxious dose of PGE_2. This finding gives new insight into long-lasting effects of chemotherapeutics on the nociceptive system and demonstrates that a variety of AMPK activators can be used to attenuate and/or reverse this type of plasticity. Since clinical CIPN can be recurring (Ewertz et al., 2015), hyperalgesic priming may be a useful paradigm for understanding how to break the cycle of pain that can occur in certain patients. Previous studies have shown that CIPN pain resolution is actively regulated by T cells and interleukin 10 (Krukowski et al., 2016; Laumet et al., 2019). Similar observations have been made in inflammatory and post-surgical pain models wherein pain resolution occurs via a mechanism that involves inhibitory G-protein coupled receptors in the spinal cord dorsal horn or nociceptive afferents (Corder et al., 2013; Taylor and Corder, 2014; Price and Inyang, 2015; Walwyn et al., 2016; Severino et al., 2018). While it is not known to what extent these pain resolution mechanisms overlap with mechanisms that resolve hyperalgesic priming, it is notable that AMPK activators are effective in reversing hyperalgesic priming in all of these contexts (Tillu et al., 2012; Burton et al., 2017; Inyang et al., 2019b).

It has previously been established that metformin is capable of blocking the establishment of CIPN as a result of paclitaxel treatment in mice but that this drug does not reverse established CIPN (Mao-Ying et al., 2014). Our findings are consistent with that previous work insofar as we saw no effect of metformin on established paclitaxel-induced mechanical hypersensitivity in male and female mice. However, we did note an inhibition of hyperalgesic priming in both sexes, indicating that metformin does have effects even when given after cessation of chemotherapy treatment. While the anti-hyperalgesic effects of metformin in male mice are very robust in the SNI and incisional models (Melemedjian et al., 2011; Burton et al., 2017; Inyang et al., 2019a), our previous observations in female mice have demonstrated a

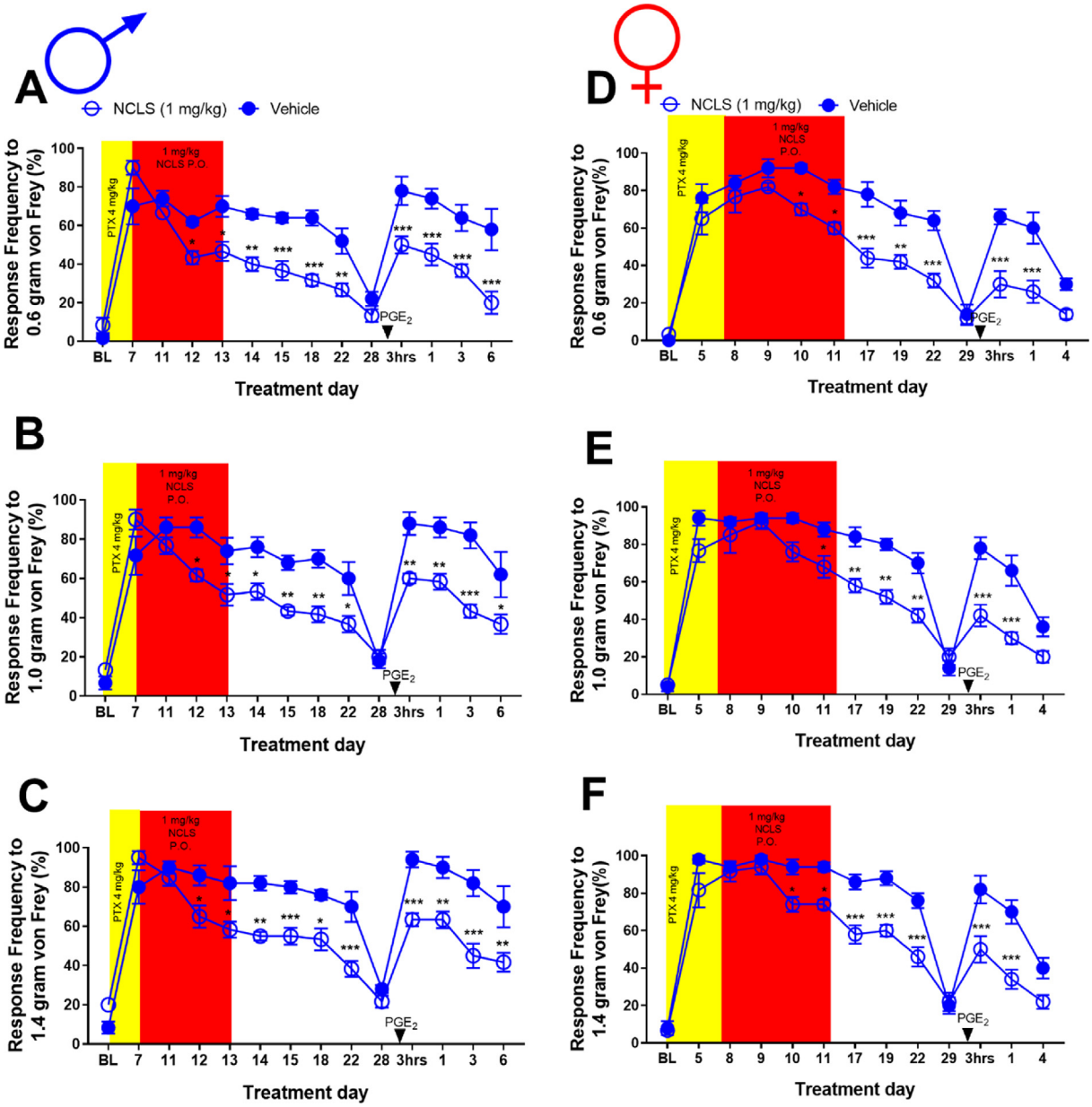

Fig. 4. Narciclasine partially reverses paclitaxel-induced mechanical hypersensitivity and blocks priming in male and females. A-C. NCLS reverses CIPN in male mice when give immediately following paclitaxel treatment and blocked hyperalgesic priming precipitated by PGE_2. *p < 0.05; **p < 0.01; ***p < 0.001; N = 6 for the NCLS group and N = 6 for the vehicle group. D-F. NCLS reversed CIPN in female mice when given immediately following 7-day paclitaxel treatment and attenuated hyperalgesic priming precipitated by PGE_2. *p < 0.05; **p < 0.01; ***p < 0.001; N = 5 for the NCLS group and N = 5 for the vehicle group.

marked sexual dimorphism in the efficacy of metformin (Inyang et al., 2019a) (Inyang et al., 2019b). Surprisingly, in this study, metformin was just as effective in reversing hyperalgesic priming in females as it was in males. While the mechanistic underpinning of these divergent sex-specific effects are not currently known, several clinical trials with metformin for pain suggest that this drug can be effective in both male and female humans (Taylor et al., 2013; Kialka et al., 2016; El-Fatatry et al., 2018). Additional clinical trials will be needed to understand the utility of metformin as a pain therapeutic. Our work provides new information that can be useful in designing those clinical trials.

Narciclasine has shown robust behavioral effects in mice in treatment of acute and chronic pain (Inyang et al., 2019b) as well as combating inflammation (Stark et al., 2019) making it an attractive potential analgesic. One benefit of narciclasine is that it is a natural compound and can be extracted as well as synthesized (Ceriotti, 1967). Compared to other AMPK activators, narciclasine stimulates the kinase *in vivo* at low doses and shows efficacy in cancer, metabolic and pain models at doses that coincide with AMPK activation kinetics (Van Goietsenoven et al., 2010; Julien et al., 2017a,b; Inyang et al., 2019b). Mechanistically, narciclasine increases the adenosine diphosphate (ADP)/ATP ratio in myotubes, which is a potential mechanism through which the compound causes AMPK activation (Hu et al., 2015; Julien et al., 2017a,b). The mechanism through which narciclasine activates AMPK in sensory neurons is not known, but our previous work demonstrates that the drug stimulates AMPK in dorsal root ganglion neurons *in vitro* and *in vivo* (Inyang et al., 2019b). Interestingly, while narciclasine activates AMPK in skeletal muscle and neurons, it does not activate AMPK in liver (Julien et al., 2017a,b; Inyang et al., 2019b). The restricted action of narciclasine on AMPK in certain tissues may be advantageous from a safety perspective. We cannot rule out a potential action of narciclasine on other signaling mechanisms. For instance, narciclasine negatively influences inflammation via downregulation of

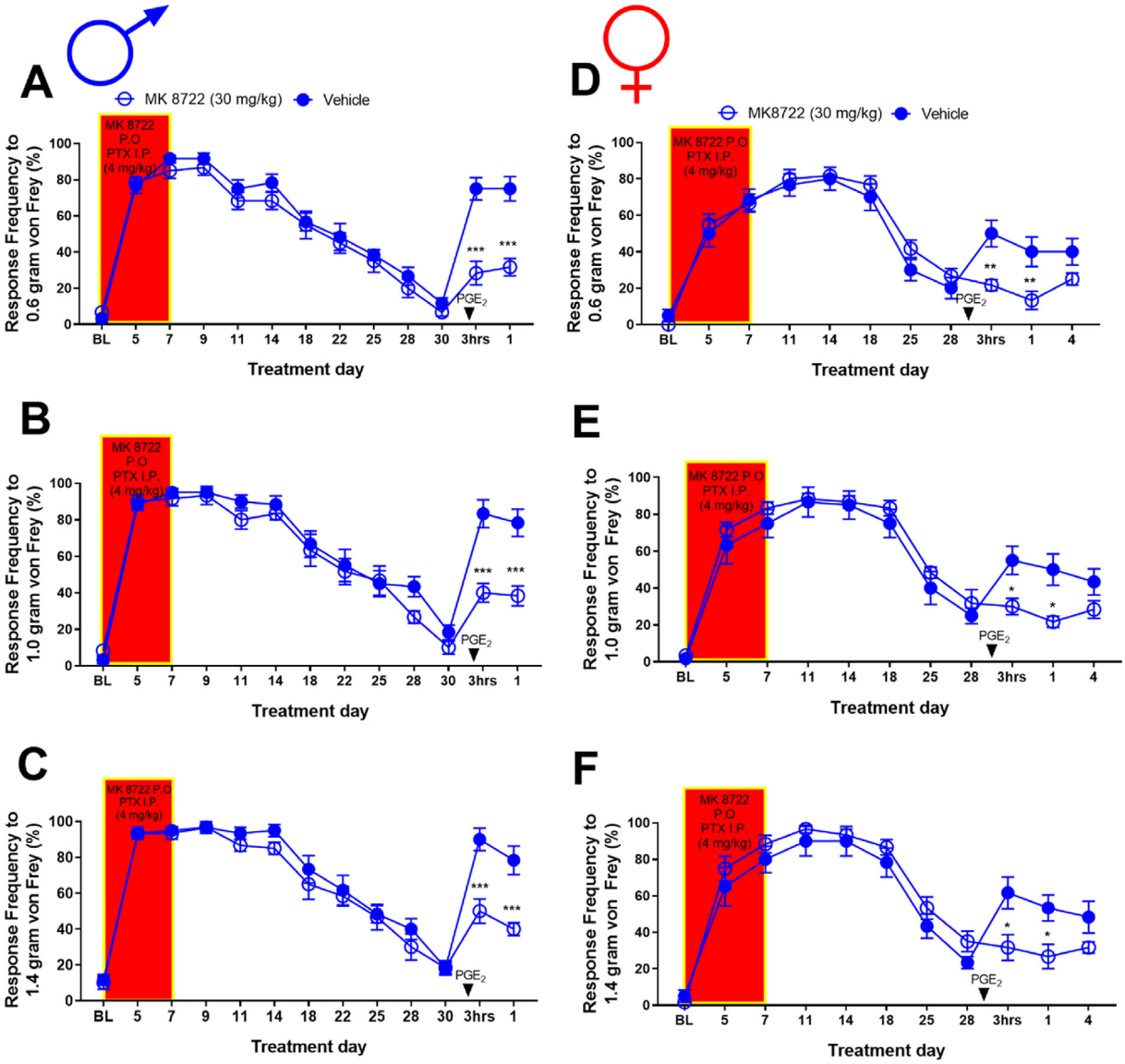

Fig. 5. MK8722 attenuates paclitaxel-induced hyperalgesic priming in male and female mice when given concurrently with paclitaxel. A-C. MK8722 did not block the development of CIPN in male mice when give concurrently with paclitaxel but was effective in attenuating hyperalgesic priming precipitated by PGE_2. ***p < 0.001; N = 6 for MK8722 group and vehicle group. D-F. MK8722 failed to prevent CIPN in female mice when given concurrently with paclitaxel treatment but again attenuated hyperalgesic priming precipitated by PGE_2. *p < 0.05; **p < 0.01; ***p < 0.001; N = 4 for NCLS and vehicle group.

tumor necrosis factor type 1 receptor (Stark et al., 2019), which would also be expected to have an impact on neuropathic pain. It is possible that engagement of multiple molecular targets by this compound improves its *in vivo* efficacy.

AMPK activators are likely effective for alleviating pain in CIPN for several reasons. One is that paclitaxel treatment induces enhanced signaling in mTOR and MAPK signaling pathways and activation of AMPK targets both of these signaling mechanisms to reduce nociceptor excitability (Megat et al., 2019). Another potential target is mitochondrial dysfunction in CIPN. This has recently been comprehensively reviewed by Trecarichi and Flatters (Trecarichi and Flatters, 2019) and is clearly a central feature of sensory neuron dysfunction across CIPN types (Ma et al., 2018). AMPK activation has multiple effects on dysfunctional mitochondria in many different contexts (Hardie et al., 2012; Burkewitz et al., 2014). However, the influence of AMPK activation on mitochondrial dysfunction in CIPN has not been addressed directly.

There are several weaknesses of this study that could be addressed in future experiments. Behavioral measures in this work relied completely on evoked pain measures. Future experiments will focus on the effect of narciclasine on affective components of pain using the conditioned place preference paradigm (King et al., 2009; Megat et al., 2019). Another shortcoming of this study is that it was completely focused on mechanical pain features of CIPN. CIPN is characterized by other features such as weakness and numbness (Izycki et al., 2016) that we did not assess. Our previous work with metformin demonstrated that numbness and the loss of intra-epidermal nerve fibers could be prevented by metformin treatment in both sexes (Mao-Ying et al., 2014). We hypothesize that narciclasine would be similarly effective, but might also reverse these effects once established, unlike our observations with metformin. Finally, and as mentioned above, the common molecular mechanism of the compounds used in this study is AMPK, but we cannot rule out other potential mechanisms of action. It is possible that the differences we observed are due to differential efficacy in AMPK activation *in vivo* for each compound, with narciclasine likely being the most efficacious; but it is also possible that differences arise because AMPK is not the sole target for these compounds.

In conclusion, we observed that hyperalgesic priming is induced by paclitaxel treatment in mice and this priming can be attenuated with

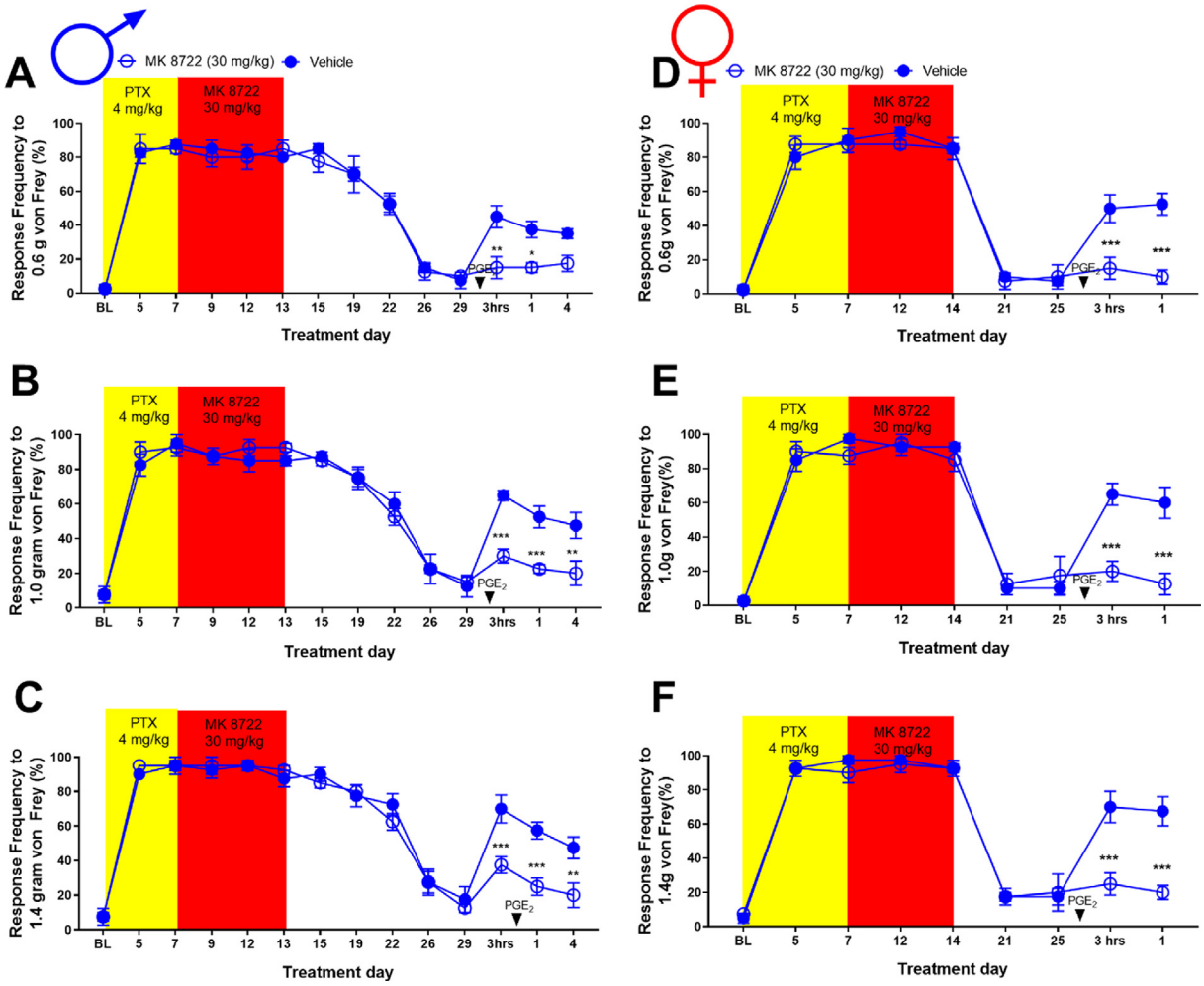

Fig. 6. MK8722 attenuates paclitaxel-induced hyperalgesic priming in male and female mice when given after paclitaxel treatment. A-C. MK8722 did not reverse CIPN in male mice when given following paclitaxel but was effective in reducing hyperalgesic priming precipitated by PGE_2. *$p < 0.05$; **$p < 0.01$; N = 6 for MK8722 group and vehicle group. D-F. MK8722 failed to reverse CIPN in female mice when given immediately following 7-day paclitaxel treatment but again inhibited hyperalgesic priming precipitated by PGE_2. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; N = 4 for MK8722 and vehicle group.

diverse types of AMPK activators in both male and female animals. We did find differential effects of these AMPK activators. Most notably, unlike metformin and MK8722, narciclasine both prevented and reversed paclitaxel-induced mechanical hypersensitivity suggesting that this compound can have a unique, disease modifying effect on CIPN. Based on these and other, previously published effects (Julien et al., 2017a,b; Inyang et al., 2019b; Stark et al., 2019), the natural product narciclasine is a promising lead for further clinical development given its substantial potency and efficacy in multiple preclinical pain models in both sexes.

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Author contributions

K.E.I, A.K., C.J.H. G.L., M.D.B. G.D. and T.J.P. conceived of the project. K.E.I, A.K., C.J.H., M.D.B., G.D. and T.J.P. designed experiments. K.E.I, T.A.M., E.D.R. and M.W. did behavioral experiments. K.E.I. and T.J.P. analyzed data. K.E.I and T.J.P. wrote the first draft of the manuscript and all authors edited the manuscript. All authors approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynpai.2019.100037.

References

Brown, T.J., Sedhom, R., Gupta, A., 2019. Chemotherapy-induced peripheral neuropathy. JAMA Oncol.

Bullón, P.A.-G.E., Carrión, A.M., Marín-Aguilar, F., Garrido-Maraver, J., Román-Malo, L., Ruiz-Cabello, J., Culic, O., Ryffel, B., Apetoh, L., Ghiringhelli, F., Battino, M., Sánchez-Alcazar, J.A., Cordero, M.D., 2016. AMPK phosphorylation modulates pain by activation of NLRP3 inflammasome. Antioxid. Redox Signal 24, 157–170.

Burkewitz, K., Zhang, Y., Mair, W.B., 2014. AMPK at the nexus of energetics and aging. Cell Metab. 20, 10–25.

Burton, M.D., Tillu, D.V., Mazhar, K., Mejia, G.L., Asiedu, M.N., Inyang, K., Hughes, T., Lian, B., Dussor, G., Price, T.J., 2017. Pharmacological activation of AMPK inhibits

incision-evoked mechanical hypersensitivity and the development of hyperalgesic priming in mice. Neuroscience 359, 119–129.
Cao, C., Huang, W., Zhang, N., Wu, F., Xu, T., Pan, X., Peng, C., Han, B., 2018. Narciclasine induces autophagy-dependent apoptosis in triple-negative breast cancer cells by regulating the AMPK-ULK1 axis. Cell Prolif. 51, e12518.
Ceriotti, G., 1967. Narciclasine: an antimitotic substance from Narcissus bulbs. Nature 213, 595–596.
Colvin, L.A., 2019. Chemotherapy-induced peripheral neuropathy: where are we now? Pain 160 (Suppl 1), S1–s10.
Corder, G., Doolen, S., Donahue, R.R., Winter, M.K., Jutras, B.L., He, Y., Hu, X., Wieskopf, J.S., Mogil, J.S., Storm, D.R., Wang, Z.J., McCarson, K.E., Taylor, B.K., 2013. Constitutive mu-opioid receptor activity leads to long-term endogenous analgesia and dependence. Science 341, 1394–1399.
Dumont, P., Ingrassia, L., Rouzeau, S., Ribaucour, F., Thomas, S., Roland, I., Darro, F., Lefranc, F., Kiss, R., 2007. The Amaryllidaceae isocarbostyril narciclasine induces apoptosis by activation of the death receptor and/or mitochondrial pathways in cancer cells but not in normal fibroblasts. Neoplasia (New York, NY) 9, 766–776.
El-Fatatry, B.M., Ibrahim, O.M., Hussien, F.Z., Mostafa, T.M., 2018. Role of metformin in oxaliplatin-induced peripheral neuropathy in patients with stage III colorectal cancer: randomized, controlled study. Int. J. Colorectal Dis. 33, 1675–1683.
Ewertz, M., Qvortrup, C., Eckhoff, L., 2015. Chemotherapy-induced peripheral neuropathy in patients treated with taxanes and platinum derivatives. Acta Oncol. 54, 587–591.
Feng, D., Biftu, T., Romero, F.A., Kekec, A., Dropinski, J., Kassick, A., Xu, S., Kurtz, M.M., Gollapudi, A., Shao, Q., Yang, X., Lu, K., Zhou, G., Kemp, D., Myers, R.W., Guan, H.P., Trujillo, M.E., Li, C., Weber, A., Sebhat, I.K., 2018. Discovery of MK-8722: a systemic, direct pan-activator of AMP-activated protein kinase. ACS Med. Chem. Lett. 9, 39–44.
Hardie, D.G., Ross, F.A., Hawley, S.A., 2012. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat. Rev. Mol. Cell Biol. 13, 251–262.
Hu, Y., Na, X., Li, J., Yang, L., You, J., Liang, X., Wang, J., Peng, L., Bi, Y., 2015. Narciclasine, a potential allelochemical, affects subcellular trafficking of auxin transporter proteins and actin cytoskeleton dynamics in Arabidopsis roots. Planta 242, 1349–1360.
Inyang, K.E., Szabo-Pardi, T., Wentworth, E., McDougal, T.A., Dussor, G., Burton, M.D., Price, T.J., 2019a. The antidiabetic drug metformin prevents and reverses neuropathic pain and spinal cord microglial activation in male but not female mice. Pharmacol. Res. 139, 1–16.
Inyang, K.E., Burton, M.D., Szabo-Pardi, T., Wentworth, E., McDougal, T.A., Ramirez, E.D., Pradhan, G., Dussor, G., Price, T.J., 2019b. Indirect AMP-activated protein kinase activators prevent incision-induced hyperalgesia and block hyperalgesic priming, whereas positive allosteric modulators block only priming in mice. J. Pharmacol. Exp. Ther. 371, 138–150.
Izycki, D., Niezgoda, A., Kazmierczak, M., Nowak-Markwitz, E., 2016. Chemotherapy-induced peripheral neuropathy – epidemiology and pathogenesis. Ginekol. Pol. 87, 293–299.
Julien, S.G., Kim, S.Y., Brunmeir, R., Sinnakannu, J.R., Ge, X., Li, H., Ma, W., Yaligar, J., Kn, B.P., Velan, S.S., Roder, P.V., Zhang, Q., Sim, C.K., Wu, J., Garcia-Miralles, M., Pouladi, M.A., Xie, W., McFarlane, C., Han, W., Xu, F., 2017a. Narciclasine attenuates diet-induced obesity by promoting oxidative metabolism in skeletal muscle. PLoS Biol. 15, e1002597.
Julien, S.G., Kim, S.-Y., Brunmeir, R., Sinnakannu, J.R., Ge, X., Li, H., et al., 2017b. Narciclasine attenuates diet-induced obesity by promoting oxidative metabolism in skeletal muscle. PLoS Biol. 15.
Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G., 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 1, 15–25.
Kialka, M., Milewicz, T., Sztefko, K., Rogatko, I., Majewska, R., 2016. Metformin increases pressure pain threshold in lean women with polycystic ovary syndrome. Drug Des., Dev. Therapy 10, 2483–2490.
King, T., Vera-Portocarrero, L., Gutierrez, T., Vanderah, T.W., Dussor, G., Lai, J., Fields, H.L., Porreca, F., 2009. Unmasking the tonic-aversive state in neuropathic pain. Nat. Neurosci. 12, 1364–1366.
Krukowski, K., Eijkelkamp, N., Laumet, G., Hack, C.E., Li, Y., Dougherty, P.M., Heijnen, C.J., Kavelaars, A., 2016. CD8+ T Cells and Endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. J. Neurosci. 36, 11074–11083.
Laumet, G., Edralin, J.D., Dantzer, R., Heijnen, C.J., Kavelaars, A., 2019. Cisplatin educates CD8+ T cells to prevent and resolve chemotherapy-induced peripheral neuropathy in mice. Pain 160, 1459–1468.
Ma, J., Kavelaars, A., Dougherty, P.M., Heijnen, C.J., 2018. Beyond symptomatic relief for chemotherapy-induced peripheral neuropathy: Targeting the source. Cancer 124, 2289–2298.
Mao-Ying, Q.L., Kavelaars, A., Krukowski, K., Huo, X.J., Zhou, W., Price, T.J., Cleeland, C., Heijnen, C.J., 2014. The anti-diabetic drug metformin protects against chemotherapy-induced peripheral neuropathy in a mouse model. PLoS ONE 9, e100701.
Megat, S., Ray, P.R., Moy, J.K., Lou, T.F., Barragan-Iglesias, P., Li, Y., Pradhan, G., Wanghzou, A., Ahmad, A., Burton, M.D., North, R.Y., Dougherty, P.M., Khoutorsky, A., Sonenberg, N., Webster, K.R., Dussor, G., Campbell, Z.T., Price, T.J., 2019. Nociceptor translational profiling reveals the ragulator-rag GTPase complex as a critical generator of neuropathic pain. J Neurosci 39, 393–411.
Melemedjian, O.K., Khoutorsky, A., 2015. Translational control of chronic pain. Prog. Mol. Biol. Transl. Sci. 131, 185–213.
Melemedjian, O.K., Khoutorsky, A., Sorge, R.E., Yan, J., Asiedu, M.N., Valdez, A., Ghosh, S., Dussor, G., Mogil, J.S., Sonenberg, N., Price, T.J., 2013. mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK. Pain 154, 1080–1091.
Melemedjian, O.K., Asiedu, M.N., Tillu, D.V., Sanoja, R., Yan, J., Lark, A., Khoutorsky, A., Johnson, J., Peebles, K.A., Lepow, T., Sonenberg, N., Dussor, G., Price, T.J., 2011. Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain. Mol. Pain 7, 70.
Pachman, D.R., Barton, D.L., Watson, J.C., Loprinzi, C.L., 2011. Chemotherapy-induced peripheral neuropathy: prevention and treatment. Clin. Pharmacol. Ther. 90, 377–387.
Price, T.J., Inyang, K.E., 2015. Commonalities between pain and memory mechanisms and their meaning for understanding chronic pain. Prog. Mol. Biol. Transl. Sci. 131, 409–434.
Qin, X., Qiao, H., Wu, S., Cheng, J., Wan, Q., Liu, R., 2018. Curcumin inhibits monocyte chemoattractant protein-1 expression in TNF-alpha induced astrocytes through AMPK pathway. Neurochem. Res. 43, 775–784.
Reichling, D.B., Levine, J.D., 2009. Critical role of nociceptor plasticity in chronic pain. Trends Neurosci. 32, 611–618.
Seretny, M., Currie, G.L., Sena, E.S., Ramnarine, S., Grant, R., MacLeod, M.R., Colvin, L.A., Fallon, M., 2014. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis. Pain 155, 2461–2470.
Severino, A., Chen, W., Hakimian, J.K., Kieffer, B.L., Gaveriaux-Ruff, C., Walwyn, W., Marvizon, J.C.G., 2018. Mu-opioid receptors in nociceptive afferents produce a sustained suppression of hyperalgesia in chronic pain. Pain 159, 1607–1620.
Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M., Cantley, L.C., 2005. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310, 1642–1646.
Song, H.H.Y., Pan, C., Deng, X., Dai, W., Hu, L., Jiang, C., Yang, Y., Cheng, Z., Li, F., Zhang, G., Wu, X., Liu, W., 2015. Activation of adenosine monophosphate-activated protein kinase suppresses neuroinflammation and ameliorates bone cancer pain: involvement of inhibition on mitogen-activated protein kinase. Anesthesiology 123, 1170–1185.
Stark, A., Schwenk, R., Wack, G., Zuchtriegel, G., Hatemler, M.G., Brautigam, J., Schmidtko, A., Reichel, C.A., Bischoff, I., Furst, R., 2019. Narciclasine exerts anti-inflammatory actions by blocking leukocyte-endothelial cell interactions and down-regulation of the endothelial TNF receptor 1. FASEB J. 33, 8771–8781.
Taylor, A., Westveld, A.H., Szkudlinska, M., Guruguri, P., Annabi, E., Patwardhan, A., Price, T.J., Yassine, H.N., 2013. The use of metformin is associated with decreased lumbar radiculopathy pain. J. Pain Res. 6, 755–763.
Taylor, B.K., Corder, G., 2014. Endogenous analgesia, dependence, and latent pain sensitization. Curr. Top. Behav. Neurosci. 20, 283–325.
Tillu, D.V., Melemedjian, O.K., Asiedu, M.N., Qu, N., De Felice, M., Dussor, G., Price, T.J., 2012. Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. Mol. Pain 8, 5.
Trecarichi, A., Flatters, S.J.L., 2019. Mitochondrial dysfunction in the pathogenesis of chemotherapy-induced peripheral neuropathy. Int. Rev. Neurobiol. 145, 83–126.
Van Goietsenoven, G., Hutton, J., Becker, J.P., Lallemand, B., Robert, F., Lefranc, F., Pirker, C., Vandenbussche, G., Van Antwerpen, P., Evidente, A., Berger, W., Prevost, M., Pelletier, J., Kiss, R., Kinzy, T.G., Kornienko, A., Mathieu, V., 2010. Targeting of eEF1A with Amaryllidaceae isocarbostyrils as a strategy to combat melanomas. FASEB J. 24, 4575–4584.
Walwyn, W.M., Chen, W., Kim, H., Minasyan, A., Ennes, H.S., McRoberts, J.A., Marvizon, J.C., 2016. Sustained Suppression of Hyperalgesia during Latent Sensitization by mu-, delta-, and kappa-opioid receptors and alpha2A Adrenergic Receptors: Role of Constitutive Activity. J Neurosci 36, 204–221.
Weihrauch, M., Handschin, C., 2018. Pharmacological targeting of exercise adaptations in skeletal muscle: Benefits and pitfalls. Biochem. Pharmacol. 147, 211–220.